

Forensic Biology Procedures Manual

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*Denotes sections of the manual approved by the Biological Screening Technical Lead

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Section 1 Chemicals and Reagents

1.1 Critical vs. Non-Critical Reagents

By definition, "critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary or casework reference samples" (FBI QAS, 2009). Reagents which are used in pre-amplification procedures directly involved in DNA extraction from forensic casework or database samples, have been deemed critical reagents to prevent unnecessary loss of sample.

"Critical reagents, as specified by the FBI DNA QA Standard 3, require testing on established samples before use on evidentiary or casework samples.

Using a positive control(s) i.e. an established sample(s) to assess the performance of a critical reagent provides a basis for the minimum acceptable performance that can be reasonably expected from that critical reagent based on results of previous testing on the established sample(s).

A critical reagent that performs below the minimum acceptable level will be rejected and not used on evidentiary or casework samples.

Critical reagents will also be tested using a negative control sample(s) or reagent blank(s) which determine if there is any contamination in the reagent being tested. If the negative control fails – i.e. contamination is detected in the test results, the reagent will be rejected and not used on evidentiary or casework samples."

The following pre-amplification DNA/Screening reagents are exempt from the critical reagent list: Nuclear Fast Red stain, Picro-indigo-carmin stain and Ethanol, anhydrous reagent grade (for sperm slide preparation).

All post-amplification DNA reagents are hereby listed as non-critical reagents. Non-critical DNA reagents need not be verified prior to use in casework.

When a reagent fails to meet the criteria for verification, the DNA Technical Manager shall be notified and an appropriate course of action will be determined. The reagent shall not be used in casework unless or until the issue has been resolved and the approval or an alternate course of action suggested by the DNA Technical Manager has been documented.

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1.2 **General Instructions**

- Use of graduated cylinders or pipettes closest in capacity to the volume of the fluids being measured is recommended.
- Chemical and reagent quantities may be adjusted to prepare more or less than the specified amount.
- All critical reagents shall be stored in sterile/autoclaved containers.
- Reagent containers are to be labeled with the following:
 - Name of reagent
 - Lot number (the date of preparation and preparer's 2 or 3 letter initials are used as the lot # for reagents prepared in-house and reagents where a lot # is not provided by the commercial vendor; i.e. 06-0104MLC would be the lot # for a reagent prepared on Jan. 1, 2006 by MLC)
 - Any necessary safety information
- If chemicals or reagents are transferred to another container, the second container shall also contain the reagent name, lot # (or preparation date) and any necessary safety information.
- One member of the DNA discipline shall be designated for purchasing of supplies and reagents.
- For Chemicals/Reagents received without a manufacturer's expiration date: Please refer to the DNA QA Manual.
- All chemicals and reagents prepared or purchased shall be logged in the reagent log maintained in the DNA laboratory.
- All newly received/prepared critical reagents and chemicals shall be verified prior to use on casework/database samples. Chemicals/reagents requiring verification should be clearly marked as such.
- Verification of a reagent that is only used as a component of another reagent is achieved by verifying the final preparation and does not need to be documented separately.
- Reagents used in the same procedure may be verified simultaneously. If the verification fails, the components will then need to be verified separately.
- Verification paperwork for DNA critical reagents is maintained by calendar year in the LIMS. Verification paperwork for screening reagents is maintained with the reagent log and archived annually.
- For verifications that include amplification and electrophoresis, the paperwork consists of the electropherograms for the positive control/reference sample(s) and negative control/blank(s). Verification results are assessed as described in the Data Interpretation section of this manual.
- For verification of RT-PCR reagents, the verification paperwork includes the standard curve, Q-PCR set-up worksheet and Q-PCR results summary page.

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- In the verification of amplification kits, the relative fluorescence units (RFU) for the known sample amplified with the new kit are compared to the results obtained with the kit currently in use to estimate the sensitivity of the new kit. This is important for adjusting the target value with the new lot of kits.
- The central log paperwork for verifications may be referenced by noting the batch in which the verification was performed.
- Upon successful verification, the reagent log shall be updated with the verification date and analyst, and the storage location for the reagent.
- When verification fails on a reagent prepared in-house, the reagent may be re-prepared and/or verification repeated. For purchased reagents/chemicals, the DNA Technical Manager shall be consulted to determine the appropriate course of action.

1.3 Chemicals and Reagents not Requiring In-House Preparation and/or Verification

Chemicals/Reagents purchased from a commercial vendor and requiring no preparation or verification prior to use in procedures or preparation of other reagents are listed below. They shall be stored as prescribed by the manufacturer and shall expire on the date provided by the manufacturer. Expiration dates are assigned as previously described, if not provided by the manufacturer, and unless stated otherwise.

- Aluminum Sulfate [solid]
- BCIP (5-bromo-4-chloro-3-indolyl phosphate) [solid]
- Citric Acid, anhydrous [solid]
- Dithiothreitol [solid]
- Ethanol, anhydrous reagent grade [liquid]
- 10X Genetic Analyzer Buffer (GAB) from Applied Biosystems [liquid]
- Concentrated Hydrochloric acid (HCl) [liquid]
- Indigo Carmine dye [solid]
- L-F500 DNA Standard [liquid]
- Nucleic Acid Fast Red [solid]
- Phenolphthalein [solid]
- PowerPlex® 16 Matrix Standards from Promega [liquid]
- Saturated Picric Acid [liquid]
- POP-4 Polymer from Applied Biosystems [liquid]
- Potassium Hydroxide [solid]
- Sodium Acetate, anhydrous [solid]
- Sodium acetate buffer solution (3M, pH 5.2) [liquid]
- Sodium Carbonate (Na₂CO₃) [solid]

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- Sodium Hydroxide pellets [solid]
- 1N or 12N Sodium Hydroxide (NaOH) [liquid]
- STMP (Sodium Thymolphthalein Monophosphate, disodium salt) [solid]
- Xylene [liquid]
- Xylene Substitute [liquid]
- Zinc [solid]

Policies and procedures regarding selection of a suitable vendor are prescribed in the laboratory Quality Assurance Manual.

1.4

NOTE:

Vendor supplied standard samples / positive control samples such as 9947A that are sent with PCR amplification kits may be discontinued or substituted at vendors' discretion. Analyst bench notes and batch central log information will indicate the identity of positive control samples used in analysis.

Kit component information in the chemicals and reagents section(s) of manuals will be updated as required when manuals are revised.

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Section 2 Screening of Biological Evidence

The laboratory does not typically examine every piece of evidence submitted for analysis. The only items routinely screened in a sexual assault case will be the samples present in the assault kit(s). Underwear, clothing or other items, such as condoms and bedding, may require screening when no probative biological material is observed/detected in the assault kit, no kit was collected, or other under special circumstances.

The probative value of an item and the amount of time between the alleged assault and the collection of the evidence should be considered when deciding if the analysis of these samples/items is appropriate. Samples present in the assault kit(s) may not be examined due to the length of time between the offense and collection. Information pertaining to the date of offense and date of examination may be found on the Forensic History Form, the Victim Information/Medical History Form or on the outside of the kit box/envelope.

2.1 Hair and Fiber Evidence

The following sexual assault kit components may need to have trace evidence evaluated/isolated before biological screening/testing can be conducted:

- Foreign Material Sheet
- Debris Collection
- Pubic Hair Combing (combing will not be examined if the victim/suspect has bathed/showered or when the sample was collected 2 or more days after the offense)
- Miscellaneous Evidence
- Underwear/Clothing

Hair and fiber evidence may be recovered from evidentiary items by the following methods:

Tape Lifting

Clear plastic latent fingerprint tape (or other suitable adhesive tape) can be applied adhesive side down to the surface of the evidence item. Press the tape down, and then pull away. Hairs and fibers will adhere to the adhesive on the tape. Place the tape adhesive side down on the shiny-side of freezer paper (or on another appropriate surface).

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Scraping

The item to be examined is suspended above the examination surface and gently scraped with a clean metal spatula. Scraping in a downward direction allows surface debris to fall onto the examination surface for collection. The debris is transferred to an appropriate storage container.

Hand-picking

Clean forceps are used to collect trace evidence from evidence items. The isolated material is then placed in an appropriate container. Isolated trace evidence should be sealed in a suitable container and clearly marked with identifying information. Isolated trace evidence may be placed within the originating item or it may be repackaged as an entirely new item of evidence.

The laboratory does not currently provide microscopic comparisons for hair and fiber evidence submitted or recovered in case work. The laboratory will screen the evidence submitted in these cases and, when needed, evidence may be sent to another laboratory for analysis. Examination of trace evidence need not occur in cases where the trace is not likely to have probative value. In these cases, trace collections are repackaged with the original evidence and the bench notes will reflect that no examination of the trace evidence was conducted.

2.1.1 Screening Hairs

An intact hair will have a root portion and a shaft portion. It is important to start with visual examination followed by macroscopic examination of the morphology of individual hairs. An initial examination at a magnification of 7X, followed by an examination at 30X or higher, will enable the analyst to record the overall shape of the hair, whether or not a root is present and, where present, its shape and appearance, the basic features of the shaft, and medullary structure.

Hairs recovered in case work will be examined visually and macroscopically, using a stereoscope, to determine the following:

- Is the hair Animal or Human in origin
- If Human, somatic region (head hair, pubic hair, inconclusive or other)
- If Human, is the hair suitable for nuclear DNA analysis

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The analyst will also document the observed characteristics of the hair that were used to make the above described determinations. These characteristics may include the following:

Animal vs. Human

- color(s) of the hair
- medullary structure
- root shape
- hair shape or form

Head Hairs

- length of hair
- moderate shaft diameter and diameter variation.
- medulla absent to continuous and relatively narrow when compared with its structure in hairs from other body areas.
- often with cut or split tips.
- may show artificial treatment (solar bleaching, dyeing, or mechanical damage)
- Soft texture (pliable).
- little or no taper.

Pubic Hairs

- shaft diameter coarse with wide variations and buckling.
- medulla relatively broad and usually continuous when present.
- follicular tag often present on the root.
- tips usually rounded or abraded.
- stiff texture (wooly).

If the analyst is unable to identify the somatic region of the hair, this should be indicated in the bench notes. Another qualified hair examiner may be consulted for assistance.

Suitable for Autosomal STR Analysis

- anagen or early catagen growth phase
- visible tissue/sheath material
- follicular tag

DNA analysis of a hair consumes a portion of the hair, preventing subsequent examinations. If the analyst concludes that the hair(s) examined may be suitable for nuclear DNA analysis, a digital image of the hair's root will be included in the analyst's bench notes.

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2.2 Vaginal/Cervical, Rectal and Oral Swabs and Smears

Samples collected within the following time frame should be examined for the presence of spermatozoa:

- Vaginal/Cervical - 7 days
- Rectal - 2 days
- Oral - 1 day

These guidelines, based on published literature, apply only to living adult female victims. Different considerations may apply to samples collected from children, adult males, and homicide victims.

Procedure

- Sample/cut a small portion of the swab(s) and follow the Extraction Protocol as described in the Forensic Biology Work Instructions.
- Stain and grade the slide(s) as described in the Forensic Biology Work Instructions. Document results in bench notes.
- Smear slides received in kits will not be stained and examined. Swabs will always be extracted and examined for the presence of spermatozoa.

2.3 Miscellaneous Evidence Swabs

Miscellaneous evidence samples such as blood, semen, saliva or DNA (contact/touch) swabs may also be collected and submitted for analysis.

Samples present in the Miscellaneous Swabs Semen envelope or any other envelope labeled as possible semen or a wood's + sample may be examined for the presence of semen/spermatozoa using methods described in the Forensic Biology Work Instructions.

Samples present in the Miscellaneous Swabs Blood envelope or any other envelope labeled as a possible blood sample may be examined for the presence of blood using methods described in the Forensic Biology Work Instructions.

Samples present in the Miscellaneous Swabs Saliva envelope, the Miscellaneous Swabs DNA envelope, or any other envelope labeled as containing a sample with possible foreign saliva or skin cells, may be suitable for DNA testing.

The laboratory does not conduct presumptive testing for the presence of saliva, urine or feces. Currently there are no confirmatory tests for the presence of these body fluids.

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2.4 External Genitalia Swabs

Swabs collected from the external genitalia area may be examined for the presence of semen/spermatozoa using methods described in the Forensic Biology Work Instructions.

Penile swabs obtained from the victim/suspect within 24 hours of the offense may be examined for the presence of blood, epithelial cells or spermatozoa (useful in cases involving multiple suspects or to help establish recent contact). The analyst will add any relevant case information to the notes section in JusticeTrax (Section 2.12).

Occasionally, external genitalia samples may be tested for foreign DNA, even when the presence of blood and/or spermatozoa is not indicated.

2.5 Fingernail Scrapings / Finger and Hand Swabs

Fingernail scrapings and finger/hand swabs may contain biological evidence such as blood, tissue, epithelial cells and spermatozoa as well as trace evidence (fibers). Victim fingernail scrapings are only examined in instances where it is believed/or indicated that the victim had scratched the assailant or in cases involving homicide. Suspect fingernail scrapings and finger/hand swabs may be useful in cases involving digital penetration.

The analyst will add any relevant case information to the notes section in JusticeTrax (Section 2.12).

2.6 Condoms

Condoms may contain evidence that can be isolated and compared to the DNA profiles of suspects or victims in sexual assault cases.

- Document the condition of the condom. Imaging may be used as needed.
- Label one pair of sterile cotton swabs "outside". Moisten the swabs using sterile deionized water. Swab the "outside" surface of the condom, as received.
- Prepare a smear from the swabs collected.
- Repeat for the "inside" surface of the condom.
- Stain and grade the prepared smears as described in the Forensic Biology Work Instructions.
- Create a new item of evidence that includes the swabs and prepared slides.

Note: Acid phosphatase/BCIP testing should not be conducted when screening condoms. The laboratory has encountered condoms containing seminal fluid/spermatozoa that tested negative using BCIP.

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2.7 Contact/Wearer Sources of DNA

Items not examined for the presence of biological evidence may be sampled by swabbing the item for contact/touch sources of DNA. Standard screening protocols apply.

- Document the item by imaging .
- Moisten a sterile cotton-tipped swab using sterile water.
- Swab the area(s) of interest (those likely to have the most contact with bare skin).
- Package and retain the isolated sample(s) for DNA analysis.

2.8 Semen Stain Analysis

The detection and identification of semen is achieved by visual examination, presumptive chemical testing for the presence of acid phosphatase, confirmation by microscopic identification of spermatozoa, and occasionally by testing for human seminal protein p30. Semen stains with spermatozoa present are typically subjected to DNA profiling in an attempt to identify the semen donor.

Suspected semen stains can be located by a number of methods, including careful visual examination, the use of tactile senses, fluorescence under alternate light sources, and chemical presumptive testing.

Visual and Tactile Examination

Most dried semen stains on cloth are detectable visually because their color is different from that of the material on which the semen has been deposited. Semen stains that have been exposed to warm, moist conditions may assume a yellow coloration due to the growth of bacteria. Seminal fluid frequently has a stiffening effect on fabric, forming a crusty stain.

Alternate Light Source Examination

Semen stains frequently fluoresce when examined with alternative wavelengths of light. They can appear as a bright fluorescence on a dark background or as a dark area when the background itself fluoresces.

Garments and other fabrics can be examined using the Omniprint™ 1000B or UV lamp. Mark the suspect stains with a pen and proceed with chemical presumptive tests and microscopic confirmatory tests.

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Chemical Examination

Semen stains can be located by detecting the presence of acid phosphatase activity. Visible stains can be lightly swabbed with a water-moistened cotton swab and tested with BCIP or STMP solutions.

Detailed instructions on the use of the alternate light source and chemical methods for the detection of semen are described in the Forensic Biology Work Instructions.

2.9 Blood Stain Analysis

Blood may be present in the form of pools, spatters, or stains. The detection and characterization of blood is achieved by visual examination and subsequent presumptive testing for peroxidase-like activity of hemoglobin. Stains testing positive to a presumptive test for blood may be further characterized by determining if the stain is animal or human in origin, or by DNA profiling to provide information as to the blood's source.

Suspected bloodstains can be located by a number of methods, including visual and stereoscopic examination, the use of alternate light sources and chemical presumptive testing.

Visual Examination

Most dried bloodstains on cloth can be detected visually because their color is different from that of the material on which the blood has been deposited. Bloodstains range in color from a reddish-brown to black.

Blood deposited on dark colored items may be difficult to locate visually. The use of an infrared hand held scope, video-imaging system using an infrared filter, or Omniprint™ 1000B may assist in the location of possible bloodstains. Use of these sources will provide the contrast needed to search for stains that are not visible otherwise.

Bloodstains on clothing and other porous substrates tend to be wicked into the substrate under dirt and debris, which will be found on and around the fibers. This can easily be seen microscopically.

Bloodstains deposited on non-porous substrates will dry on the surface as a film or a crust.

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Chemical Examination

Suspected blood stains can be tested for peroxidase-like activity with Phenolphthalein reagent and hydrogen peroxide. Stains can be sampled with a dry or moistened swab or piece of filter paper, or by teasing a few stained fibers from the substrate.

Stains testing positive to this presumptive test for blood may be further characterized as to their origin (human or higher primate) using Human Hemoglobin analysis by immunoassay.

Detailed instructions on the use of the alternate light source and chemical methods for the detection of blood are described in the Forensic Biology Work Instructions.

2.10 Processing, Documentation and Isolation of Biological Stains

General Instructions

- Do not allow one evidence stain to come into contact with other biological samples and do not collect or package two separate stains together.
- Change paper between items or between pertinent groups of items (i.e., suspect and victim or items from different crime scenes).
- When possible, isolate and manipulate stains with sterile, disposable implements. When non-disposable tools are used, they can be cleaned by thoroughly rinsing with a stream of distilled water and drying with paper tissue, or by rinsing with ethanol and flaming. This process should be repeated twice before using the tool to manipulate another sample.
- Small biological stains (i.e., 2mm size bloodstain) and items suspected of containing minimal stains should be handled with clean gloves and before items containing larger stains.
- It is important to save as much sample as possible to permit re-analysis at a later date, if needed. Presumptive and/or confirmatory testing methods should not be performed if the testing would consume the entire sample or would prevent re-analysis by the crime lab or any outside laboratory/agency.
- Received items are labeled with the case and item numbers, and the analyst's initials and date of examination.
- Items of evidence are examined, one at a time, on clean laboratory paper. Take care, when opening items, to minimize unnecessary destruction of the packaging or previous seals.
- Where possible, the item itself should be labeled away from stained areas.

Note: Exercise caution in labeling articles that will be submitted for latent print examinations so that any latent print evidence is not obscured or obliterated.

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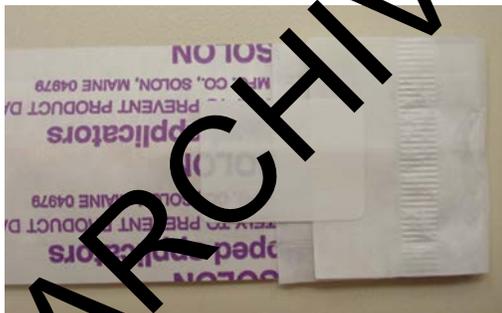
Additionally, a latent fingerprint analyst should be consulted prior to conducting any testing on these items.

- If any previous examination/testing is apparent or known, record the nature of the test and the results obtained. Identify field-tested stains as “possible” blood or semen stains.
- Take detailed notes describing each item and its packaging. When practical, digital images of items should be included in the bench notes.
- Collect other trace evidence that may be present and of possible forensic significance.
- Isolate stains that have tested positive to a presumptive test for blood, the presence of spermatozoa, or PSA activity for further analysis. If the stain is large, only a generous portion needs to be isolated. The portion of the stain sampled should be documented in the bench notes.

2.11 Packaging of Isolated Stains

Place dried swabs back in their original package(s) or a swab carton. Place any isolated cuttings into a glassine envelope. Secure the package(s). (**Figure 1**). Evidence tape should not be used for internal packaging.

Figure 1



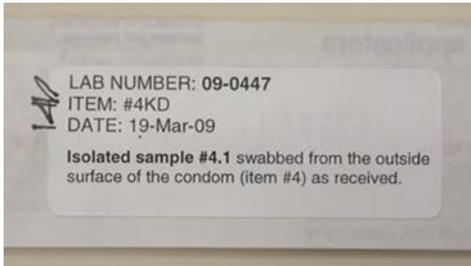
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Label each swab package, swab carton, or glassine envelope with the lab number, unique identifier (item # / your initials), a brief description / isolated stain number, and the analyst's initials along the edge of the label (**Figure 2**). The required information may be on a printed label or may be handwritten on the package.

Figure 2



Isolated stains/samples are then placed into an envelope and sealed with evidence tape. Label each envelope with the lab number, unique identifier (item #), a brief description, your initials, a biohazard label (when appropriate), and a LIMS barcode .

The required information may be on a printed label or may be handwritten on the package. Individual glassine sleeves containing separate samples from the same item can be placed together into one larger envelope; as can individual swab packages from a single item (i.e. swabs from inside/outside of condom). Stains/samples from separate items are packaged in separate envelopes.

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Figure 3



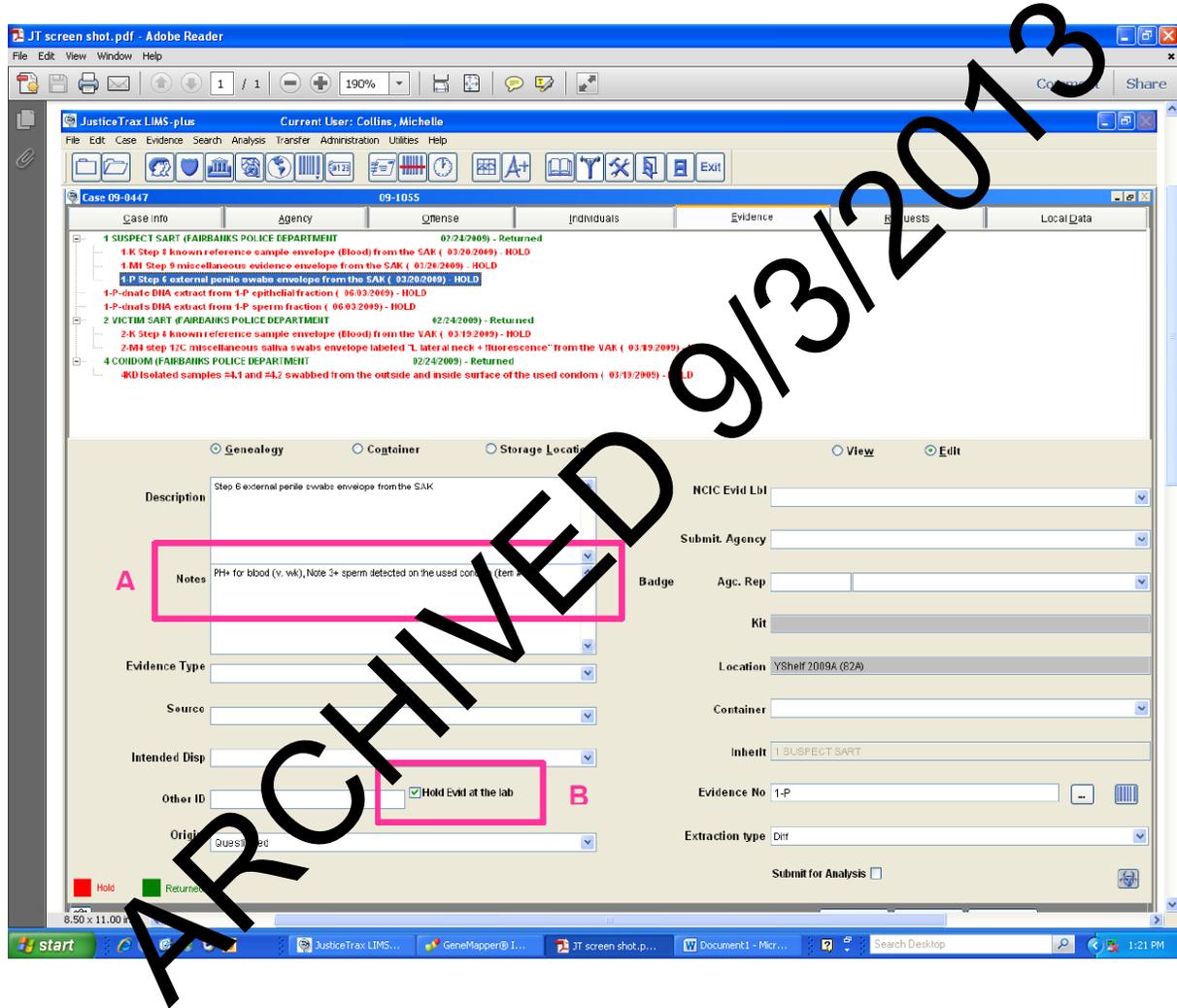
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In the “Notes” section in JusticeTrax (A) document any relevant case/sample information as needed (Figure 5). Example: sperm 2+ detected on the vaginal smear, victim had consensual intercourse with boyfriend the day before the offense, etc.

Figure 5



Mark all items to be retained for DNA analysis in JusticeTrax by checking the box labeled “Hold Evid at the lab” (B). Typically, entire assault kits will be retained by the laboratory and itemization of contents in LIMS is not necessary.

Transfer the evidence to the tote (if applicable) and return it to the evidence section of the laboratory.

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Section 3 DNA Extraction

3.1 General instructions

- DNA extractions are performed in a designated laboratory, with equipment dedicated to the DNA extraction work area.
- Analysts will use a clean cutting surface and sterile consumables for sampling items of evidence. Non-disposable items used for evidence handling, such as hole punches, will be cleaned before and after use by repeated punching of clean paper.
- Questioned samples and reference samples will be extracted separately in time and/or location.
- Extraction order is planned so that samples with potentially high levels of DNA are processed after samples with potentially lower levels of DNA.
- Sample tubes must be centrifuged before opening and only one sample tube is to be open at a time.
- DNA extraction information is documented on the designated worksheet. This worksheet must include the batch name, a description of the item, extraction procedure used (e.g. EZ1 Trace or Large Volume (LV) protocol for casework samples), extraction instrument, sample size, elution volume (for casework extractions), and the date extraction is started (e.g. the date the evidence is cut/sampled). If the questioned samples and reference samples are extracted on the same day, the time when each set of extractions was begun is documented.
- Each batch of extractions must include appropriate reagent blanks for each type of extraction performed. All reagents (lot# and expiration date) and reagent blanks in a batch of samples are documented on the designated worksheet.
- The volumes specified in the extraction work instructions are suitable for most forensic stains and reference samples. These volumes may be adjusted appropriate to the size and nature of the sample(s).
- Digital imaging of evidence
 - It may be appropriate for a DNA analyst to image an item of evidence that has not previously been examined and documented by another discipline in the laboratory (for example, a cigarette butt). These images should include a scale, and are labeled with the case #, item #, date and analyst's initials, and will be included in the bench notes

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- Guidelines for the workflow between Biological Screening analysts and DNA analysts for processing evidence in sex assault cases (and proficiency tests):
 - DNA analysts do not need to repeat presumptive blood tests performed and reported in a biological screening report.
 - DNA reports do not need to report the results of microscopic examinations previously issued in a biological screening report. Note: if the biological screening report contained only the results of smears from an assault kit and the analyst is processing the associated swabs, the DNA report should contain the results of slides prepared during DNA extraction.
 - The slide is retained with the item when microscopic results are being issued in the DNA report. Otherwise, slides may be discarded upon completion of the case technical review.
 - A DNA analyst who is not competent in microscopic examinations must get a second read of the slide by a qualified analyst (when the microscopic results will be issued in the DNA report). This is documented on the extraction worksheet and by the qualified analyst's initials on the retained slide.
 - For items not routinely screened prior to DNA analysis (e.g. penile swabs, fingernail scrapings), the DNA analyst should check the evidence item record in LIMS for notes entered by the biological screener, indicating the possible presence of spermatozoa in the samples that were not screened.

3.2 Sampling Items for DNA Analysis

Note: Whenever feasible, a portion of the original evidence will be retained to provide an opportunity for re-testing. If the entire original sample is consumed in extraction, the total elution volume must be at least 100 μ L. When there is a known suspect, and an analyst determines that the entire original sample and DNA extract must be consumed in analysis to give the best chance for obtaining a result, the analyst shall obtain written permission from the District Attorney's Office prior to consuming the DNA extract. This is not required for unknown suspect cases.

3.2.1 Bloodstains

- Typical bloodstains on fabric are sampled by cutting approximately 0.5sq cm of the stain using a sterile, disposable scalpel. The size of the cutting may vary depending on the size and condition of the stain. The entire stain may be sampled for very small or potentially degraded stains.
- Bloodstains on thick fabric or filter paper may be cut, or sampled using a 3mm punch.

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- Bloodstains may also be sampled by swabbing with a damp, sterile swab if the substrate is difficult to cut, or potentially contains PCR inhibitors (e.g. cigars or denim).
- Bloodstained swabs are usually sampled by cutting a portion of the stained area of the swab.

3.2.2 Swabs

- Swabs without visible staining are generally sampled by cutting approximately half of the swab lengthwise.
- Any swab(s) that is sampled should be tagged with a label containing the case number and item number.
- Typically, contact DNA swabs (not thought to contain biological fluids) will be sampled in their entirety (and eluted in at least 100 μ L), maximizing the chance of obtaining an interpretable DNA profile. When an entire sample is extracted, at least half of the extract must be retained for future use.
- Typically, known buccal swabs require only 1/3 of one swab or a small portion of two swabs.

3.2.3 Fingernail Swabs/Scrapings

- Samples are received either as swabs or debris that is scraped into a paper bindle.
- Swabs from under the nails are generally sampled by cutting the swab tips into the sample tube. However, as in the case of contact swabs, the entire swab may be used in with at least half of the extract being retained for future use.
- When sampling debris in a paper bindle, a moistened swab is used to swab the scraper, visible debris and the inside of the paper bindle. Typically one swab is used per hand. The entire swab is then cut into the sample tube. When the bindle contains a large amount of debris, a representative amount may be sampled.
- Any extract remaining after analysis should be retained since these samples may not be homogeneous.

3.2.4 Fingernails or Fingernail Clippings

- Fingernails or fingernail clippings should be imaged prior to sampling.
- Swabs from the nail(s) may be collected for profiling of tissue and cellular debris external to the nail itself. The entire swab tips are cut into the sample tube.
- If surface material, i.e. blood or dirt, is observed on the nail, the nail may be washed by immersing in 200 μ L of dilute G2 buffer (1:1 solution of H₂O:G2 buffer) in a microcentrifuge tube with gentle agitation. This may be appropriate when attempting to obtain a DNA profile of the surface material separately from the nail itself.

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- The washing is then transferred to a new microcentrifuge tube and may be extracted by adding 10 μ L Proteinase K.
- Any clippings that remain after digestion are re-packaged with the evidence.

3.2.5 Hairs

Note: Prior to DNA analysis, hair evidence shall be examined by a qualified Biological Screening analyst to determine suitability for DNA analysis.

For mounted hairs, score the edge around the cover slip with a sterile scalpel. Remove the cover slip by prying it off or by soaking the slide in xylene. Use a pipette to wash the mounting medium away with xylene. Pick up the hair with clean forceps and wash in a beaker, on a watch glass, or on a microscope slide with absolute ethanol, then wash the hair with sterile, de-ionized water in a clean container.

Note: Process a reference (known) hair sample alongside the questioned hair sample(s) as an internal control. Processing of the control hair should include shaft and root samples.

- Examine the hair, microscopically if necessary, for the presence of surface materials (i.e. blood or dirt) and document the findings. The hair may be placed on a clean piece of white paper or on a microscope slide for examination.
- If surface material is observed on the hair, the hair should be washed by immersing in 200 μ L of dilute G2 buffer in a microcentrifuge tube with gentle agitation. This may be appropriate when attempting to obtain a DNA profile of the surface material separately from the hair itself.
- The washing is then transferred to a new microcentrifuge tube and may be extracted by adding 10 μ L Proteinase K.
- Cut approximately 1cm of the proximal (root) end of the hair for digestion. Using clean forceps, place the hair root into a sterile 1.5mL tube.
- Cut approximately 1cm of the shaft adjacent to the root for separate analysis as a substrate/shaft control. Add the shaft portion of the hair to a sterile 1.5mL tube.
- The remaining portion of the hair is re-packaged with the evidence.

3.2.6 Other tissue samples

Refer to the EZ1 DNA Investigator handbook for instructions on the appropriate sample size based on tissue type. The extraction procedure is as for other sample types.

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Section 4 DNA Amplification

4.1 General instructions

- PCR set-up is performed in a designated area in the laboratory, with equipment dedicated to the work area.
- Centrifuge all sample tubes before opening and have only one sample tube open at a time.
- If reference samples and questioned samples are being set-up for amplification on the same day, always set-up questioned samples before reference samples and on a separate 96-well plate; the time when the amplifications were begun should be documented on the designated worksheet.
- The amplification worksheet will include the sample well designations, sample codes, sample concentrations (if applicable), volumes of sample amplified, and all amplification reagent lot numbers and expiration dates.
- Each amplification plate must include a positive and negative amplification control.
- Always add template DNA to the sample well/tube after addition of the PCR master mix.
- The thermal cycler(s) should be turned on prior to setting up samples to allow for time to warm up.

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4.2 Cycling Parameters on the AB GeneAmp® PCR System 9700 thermal cycler

4.2.1 PowerPlex 16 Cycling Parameters for Samples to be Analyzed on the AB 3130xl [user (alaska) and program (pp16-new)]

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 22 cycles, then:

60°C for 30 minutes
4°C hold

4.2.2 PowerPlex 16 Cycling Parameters for Database Samples to be Analyzed on the AB 3500xl [user (alaska) and program (pplex16-28 cycles)]

95°C for 11 minutes, then:

96°C for 1 minute, then:

ramp 100% to 94°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 10 cycles, then:

ramp 100% to 90°C for 30 seconds
ramp 29% to 60°C for 30 seconds
ramp 23% to 70°C for 45 seconds
for 18 cycles, then:

60°C for 30 minutes
4°C hold

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Section 5 Data Analysis with GeneMapper™ ID-X

References: GeneMapper™ ID-X Software User Guide
GeneMapper™ ID-X Software Tutorial
GeneMapper™ ID-X Software User Bulletin

The settings for the Analysis Methods are viewed by selecting GeneMapper™ ID-X Manager under the Tools drop-down menu, then clicking on the Analysis Methods tab then double clicking to select a particular Analysis Method. A range of Analysis Methods are available to analyze casework and database data. Options for Analysis Methods vary by RFU and analysis range. These methods should not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

5.1 Analysis Methods**5.1.1 Casework Analysis Methods**

25RFU Casework-2400 50RFU Casework-2400
25RFU Casework-2800 50RFU Casework-2800
25RFU Casework-3200 50RFU Casework-3200

100RFU Casework-2400
100RFU Casework-2800
100RFU Casework-3200

50RFU red Casework-2800
Blank Casework

5.1.2 Database Analysis Methods

75RFU Database-2400 100RFU Database-2400
75RFU Database-2800 100RFU Database-2800
75RFU Database-3200 100RFU Database-3200

150RFU Database-2400 250RFU Database-2400
150RFU Database-2800 250RFU Database-2800
150RFU Database-3200 250RFU Database-3200

Blank Database Global

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5.2 Allele Tab Settings

The Allele Tab Settings are viewed by opening a selected analysis method.

5.2.1 Casework Allele Tab Settings

These settings are consistent within all casework methods.

Analysis Method Editor

General **Allele** Peak Detector Peak Quality SQ & GQ Settings

Bin Set: Promega_Bins_IDX_alpha

Use marker-specific stutter ratio and distance if available

Marker Repeat Type:		Tri	Tetra	Penta	Hex
Global Cut-off Value		0.0	0.0	0.0	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	3.25	4.25	0.0
	To	0.0	4.75	5.75	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Amelogenin Cutoff		0.0			

Range Filter... Factory Defaults

Save As Save Cancel Help

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5.2.2 Database Allele Tab Settings

These settings are consistent within all database methods.

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.0	0.0	0.0	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.2	0.2	0.0
Global Minus Stutter Distance	From	0.0	3.25	4.25	0.0
	To	0.0	4.75	5.75	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Amelogenin Cutoff		0.0			

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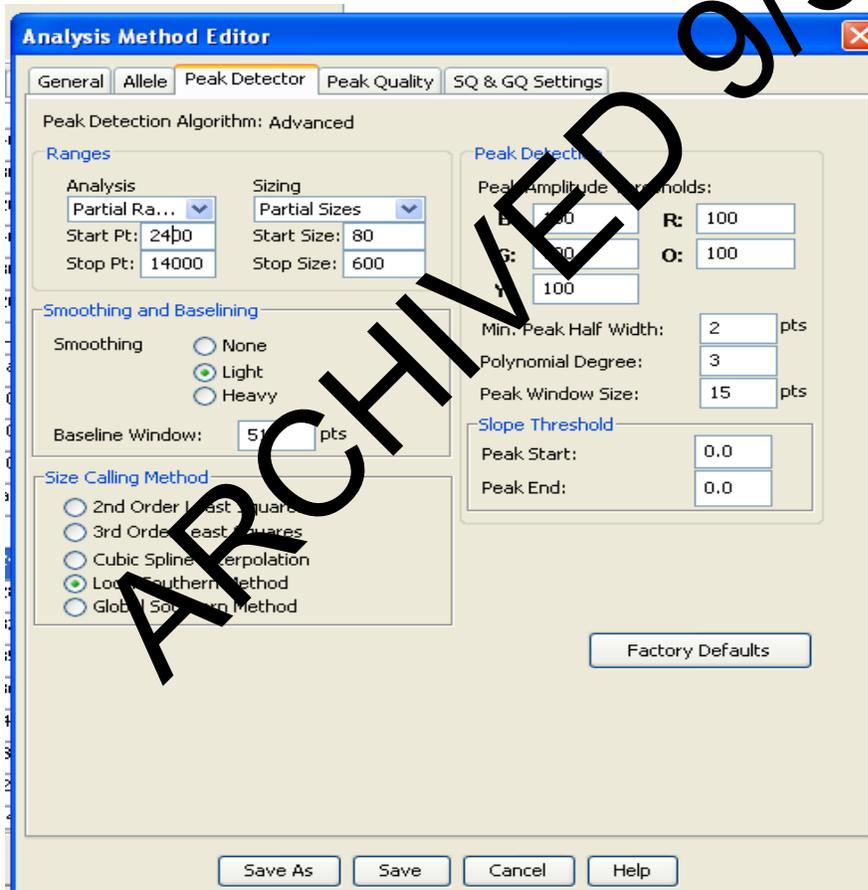
5.3 Peak Detector Tab Settings

The Analysis Range and/or the Peak Amplitude Thresholds are the only settings that vary between the alternative Analysis Methods. The Analysis Range is selected to ensure capture of the 80bp ILS peak for all allelic ladders, positive controls, and questioned and known samples in the project. The Analysis Range is selected to capture the primer peaks for reagent blanks, negative amplification controls, and samples that do not yield at least a partial profile.

5.3.1 Casework Peak Detector Tab Settings

Typically, the Peak Amplitude Threshold for all analyses is 100RFU. The use of a threshold other than 100RFU will be documented on the electropherogram(s) and must be approved by the DNA Technical Manager or CODIS Administrator.

Typical 100RFU Casework Analysis Method



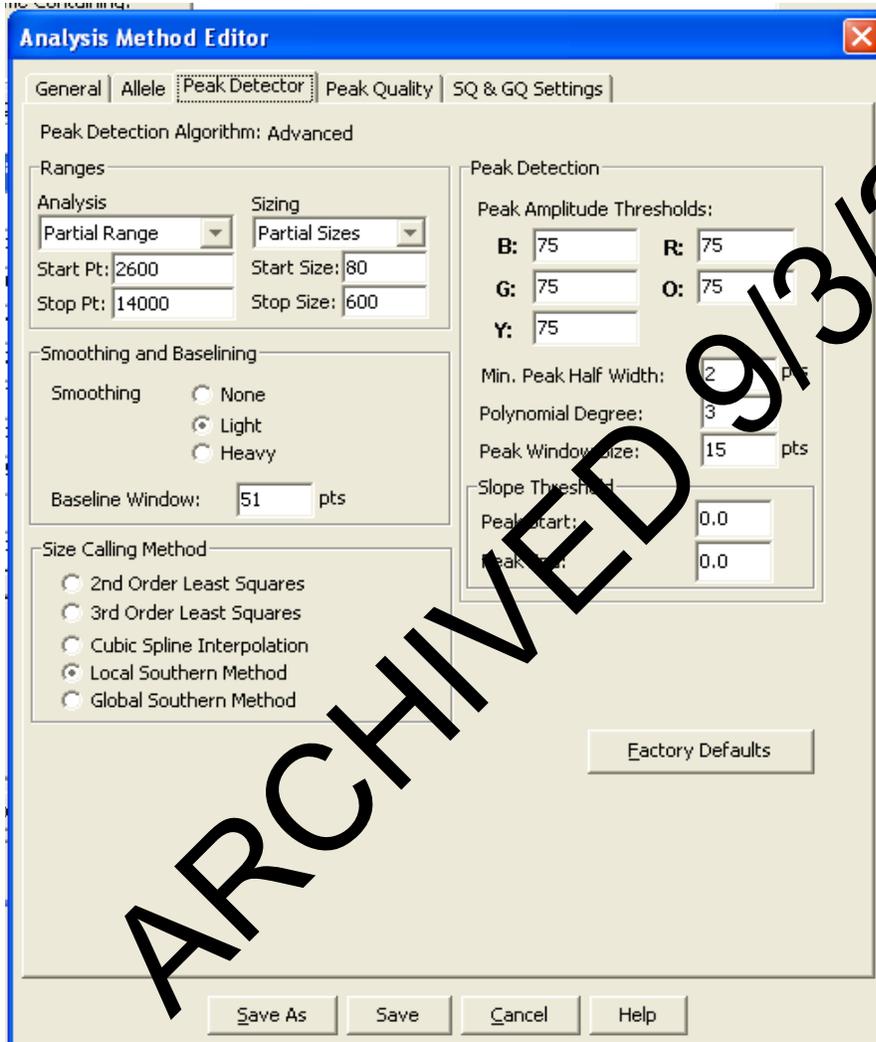
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5.3.2 Database Peak Detector Tab Settings

Peak Amplitude Thresholds between 75RFU and 250RFU are approved for database analyses.

Typical 75RFU Database Analysis Method



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5.4 Peak Quality Tab Settings

These settings do not vary among the casework methods or among the database methods, and they are not relevant in analysis of any samples where the sample type is set to Negative Control.

100RFU Analysis Methods for Casework

Analysis Method Editor

General | Allele | Peak Detector | **Peak Quality** | SQ & GQ Settings

Min/Max Peak Height (LPH/MPH)

Homozygous min peak height	150.0
Heterozygous min peak height	100.0
Max Peak Height (MPH)	8000.0

Peak Height Ratio (PHR)

Min peak height ratio	0.6
-----------------------	-----

Broad Peak (BD)

Max peak width (basepairs)	5
----------------------------	---

Allele Number (AN)

Max expected alleles	10
----------------------	----

Allelic Ladder Spike

Spike Detection	Enable
Cut-off Value	0.2

Factory Defaults

Save As | Save | Cancel | Help

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75RFU Analysis Methods for Database



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5.5 SQ and GQ Tab Settings

These settings do not vary among any of the analysis methods, and they are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

All Analysis Methods

Analysis Method Editor

General | Allele | Peak Detector | Peak Quality | **SQ & GQ Settings**

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

Broad Peak (BD)	0.7	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.7	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.7	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.3
		Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to controls)

SQ Weighting

Broad Peak (BD)	0.5
-----------------	-----

Allelic Ladder GQ Weighting

Spike (SSPK/SPK)	1	Off-scale (OS)	1
------------------	---	----------------	---

SQ & GQ Ranges

	Pass Range:	Low Quality Range:	
Sizing Quality:	From 0.75 to 1.0	From 0.0 to 0.25	
Genotype Quality:	From 0.75 to 1.0	From 0.0 to 0.25	

Reset Defaults

Save As | Save | Cancel | Help

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5.6 Data Retention

Reference: DNA Quality Assurance Manual

Raw data files will be retained at the laboratory.

GeneMapper ID-X project files are not retained long term and may be deleted after the technical and administrative reviews of a batch are completed.

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Section 6 STR Data Interpretation

Interpretation of casework and database samples is based on the guidelines contained in this section, as well as the qualified DNA analyst's training and experience, experimental results, and case specifics. In all cases, interpretations must be independently reviewed and verified based on established peer review procedures. The DNA Technical Manager may authorize the interpretation/reporting of data outside of the criteria defined herein and will document this approval by initialing and dating comments on the relevant electropherogram(s).

Casework STR Data Interpretation:

- **Reportable** alleles are those alleles whose peak heights are equal to or above the 100RFU reporting (analytical) threshold and are generally reproducible when re-amplified. Such alleles are routinely used in making comparisons between known and questioned sample genetic profiles under the interpretation guidelines stated in this manual.
- **Observed** genetic data includes data (peaks) below the reporting (analytical) threshold (100RFU) and/or alleles whose peak heights are greater than 100RFU, but that are not reproducible in replicate amplifications of the same DNA extract. Such alleles are not routinely included in the STR results table. **Observed** alleles may be considered for use when reporting the presence of multiple sources of DNA in a sample and/or when reporting the presence of male DNA. On occasion, depending on the quality of the data and the nature of the sample, such alleles may be used to generate a composite profile, with the documented approval of the DNA Technical Manager.

Database STR Data Interpretation:

The minimum peak height acceptable for database sample STR loci alleles is 75RFU. The threshold may be raised to a maximum of 250RFU for samples with an elevated baseline. **Reportable** alleles are those whose peak heights are equal to or above the 75RFU threshold. Lower threshold limits for hit confirmations or ILS (Internal Lane Standard) may be authorized by the DNA Technical Manager.

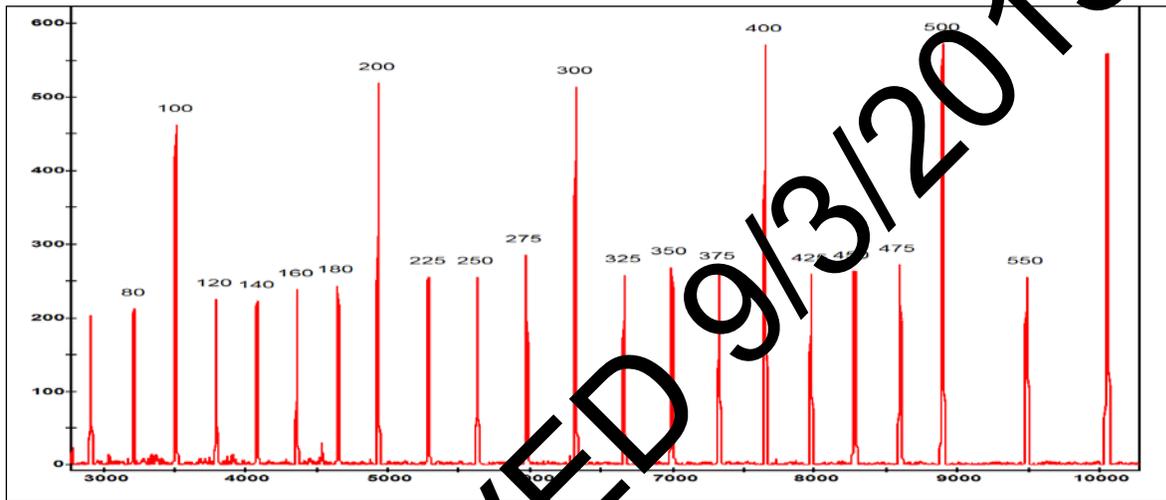
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6.1 Interpretation of the Internal Lane Standard and Allelic Ladders

6.1.1 Internal Lane Standard (ILS)

The first step in interpreting data from a run is to assess the ILS for each sample (including all controls and allelic ladders). The Promega PowerPlex16® System uses ILS 600. The analyst should verify that all peaks from 80-550 base pairs are present and labeled as shown.



The peak heights for the ILS peaks must be at least 100RFU (relative fluorescence units) to be identified and labeled by the GeneMapper™ ID-X software with a casework analysis method and 75RFU to be labeled with a database method.

When the peaks are not labeled as shown above, the analyst must determine the cause for the incorrect labeling. If the peaks at either the low or high ends are not visible, the data may be re-analyzed after changing the analysis range in the Analysis Method.

If a peak is not labeled because it falls below the reporting threshold, the analyst may, with the documented approval of the DNA Technical Manager, lower the acceptable threshold for the ILS peak(s). The Technical Manager may also approve use of samples when one of the ILS peaks (between 80 and 550bp) is not labeled. Approval is documented on the electropherogram by the Technical Manager's initials and date of approval.

Use caution when allowing for lower ILS peak heights since sample peaks may also fall below the reporting threshold when ILS peaks do. The analyst may re-inject the sample and/or set up an additional plate with the same amplified product in order to obtain

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proper ILS sizing. On occasion, adding more ILS to the sample well and re-injecting may be sufficient to achieve this.

Refer to the GeneMapper™ ID-X Software User Guide for details on manually labeling the size standard.

Database

The quality of the ILS should be noted when proper labeling of ILS peaks between 80-550bp cannot be achieved or when the SQ is less than or equal to 0.5.

6.1.2 Allelic Ladder

When the analyst has verified the correct labeling of the ILS for the samples in a run, the next step is to verify that the peaks in the allelic ladder(s) are labeled correctly. The GeneMapper™ ID-X software uses the allele calls of the ladder to assign allele calls to all the other samples in the project. The allele calls for the Promega PowerPlex16® System allelic ladder are shown below:

The allelic ladder contains the most common alleles determined for each of the 13 FBI STR core loci, the Penta D and Penta E loci, and Amelogenin. In addition, alleles not labeled in the allelic ladder (virtual alleles), may be detected and labeled in some of the samples analyzed.

At least one allelic ladder must be included in a GeneMapper™ ID-X project with the sample type designated as "allelic ladder". All the peaks in the allelic ladder must be labeled correctly for the software to assign the correct allele calls to samples in the run. When more than one ladder in a project has the sample type designated as "allelic ladder", the software will average the allelic ladders to make the allele calls for the samples in the project.

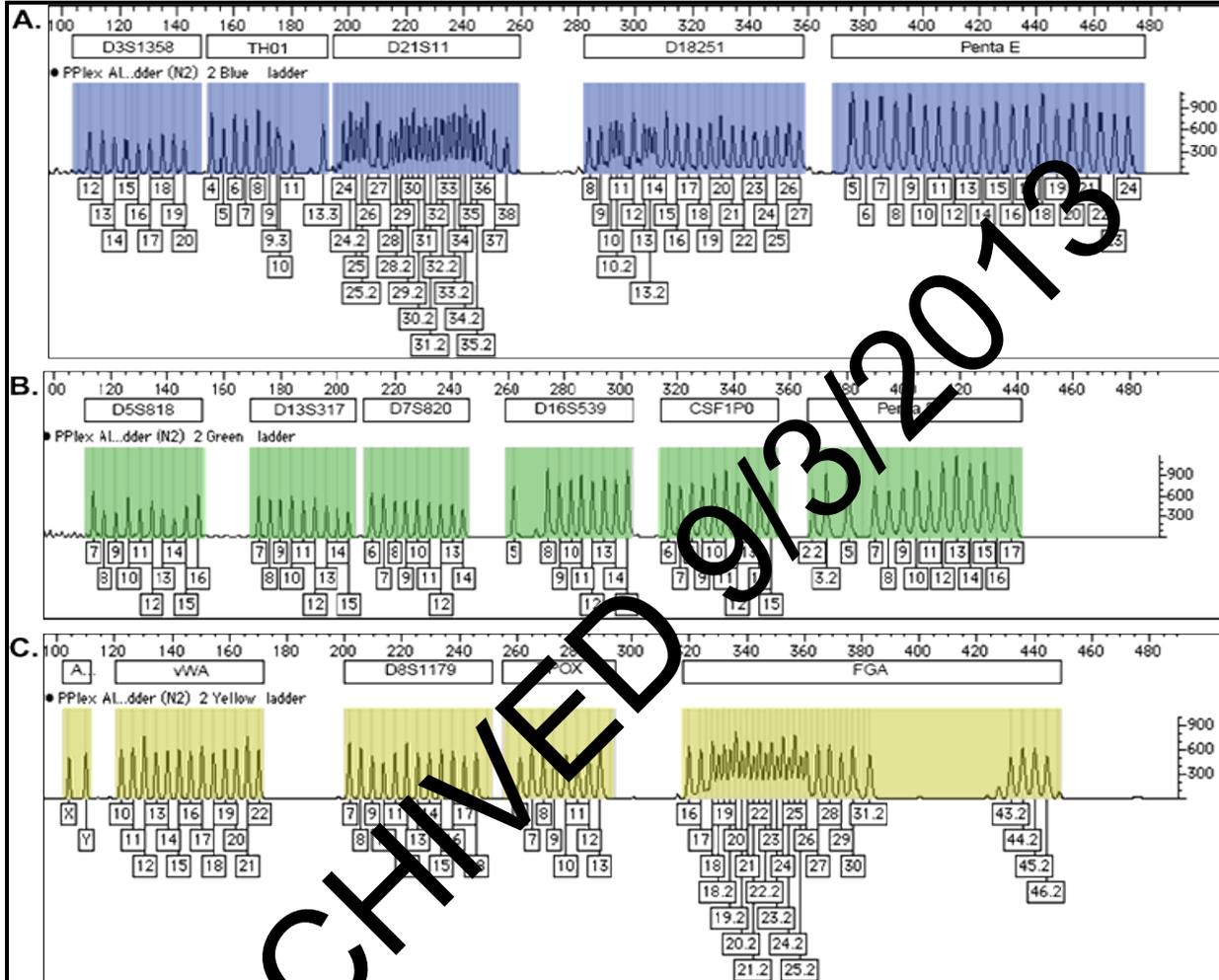
Artifacts such as spikes or "pull-up" peaks may be present that are labeled as off-ladder (OL) peaks in the allelic ladder. If all the true allele peaks are labeled correctly, such OL peaks will not affect the sample allele calls. If a spurious peak (not a true allelic peak) is labeled as a true allele (i.e. it falls within an allele size range and is designated and labeled as an allele) at any particular locus, the remaining allele designations / labels at the locus shift and will impact the correct allele call / labeling of the samples in the project. Allelic ladders with such artifacts shall not be used to analyze data in the project.

Additional information about the allelic ladder can be found in the Promega PowerPlex16® System Technical Manual.

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PowerPlex16® Allelic Ladder



6.2 Interpretation of Amplification Controls and Reagent Blank Samples

The validity of the STR data obtained for any amplification and run is dependent on obtaining acceptable results for the reagent blanks and amplification controls associated with the forensic samples. Failure to obtain clean blanks and the correct type for the positive amplification control requires careful assessment of the data in that batch of samples. All or part of an extraction, amplification and run may need to be repeated depending on the results of the controls/blanks.

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The Corrective Action Report Batch Details form (found at the back of this document) is used in batches where failed controls require Technical Manager input on the release of specific cases.

Note: Although appropriate quality assurance practices are stringently applied and enforced, it is not unexpected that low-levels of adventitious DNA (i.e., levels that result in peak heights for allelic data that are below the Laboratory's reporting threshold) attributable to staff scientists and/or consumable products directly involved in the analytical steps of such a highly sensitive technique may be detected.

6.2.1 Positive Amplification Control

The standard DNA template 9947A is currently used as the positive amplification control for the Promega PowerPlex16® System. The positive control is used to evaluate the performance of the amplification and typing procedures.

If the incorrect STR profile is obtained for the positive control, the analyst should verify that the ILS and allelic ladder peaks are labeled properly. If the ILS and allelic ladder are labeled correctly, but one or more of the alleles in the positive amplification control are labeled incorrectly or labeled as "OL" then the analyst when appropriate may remove a ladder(s) from the project or create different projects for different injections in an order to obtain acceptable allele calls for the positive amplification control. If the allele peak heights of the positive control fall below the reporting threshold, but the other samples in the batch have acceptable allele peak heights, then the positive control sample data may be analyzed at 500 FU to verify allele calls with the technical manager's approval.

Alternatively, other samples in a batch may serve as a positive control (e.g. duplicate offender/staff sample, offender confirmation sample, casework known sample with a verified profile). Use of an alternate positive control in a casework batch must have documented approval of the Technical Manager on the electropherogram.

All casework samples in a batch will be re-analyzed if only one positive control sample was used in that batch and this control fails. The laboratory will routinely include at least 5 positive control samples in every casework batch in order to prevent reanalysis of samples that are limited in DNA content. If at least one of these 5 positive control samples produces acceptable and expected results, the batch will not be reanalyzed. A positive control will be defined as a single source sample whose genetic profile was previously determined and from which a full profile was developed. The positive control

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may be a previously tested convicted offender sample or a buccal swab freshly obtained from laboratory personnel whose genetic profile(s) was/were previously determined and documented.

If all interpretable results agree for the samples between the first and second amplification/run, and the positive amplification control yields a correct STR profile for the second amplification/run, then the STR data obtained in the initial amplification/run may be used.

STR profile of 9947A



6.2.2 Negative Controls

A reagent blank is carried through the entire analytical process as part of each extraction or type of extraction. It contains all the reagents - except DNA template - used during extraction, amplification and typing.

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The negative amplification control contains only the PCR master mix (reagents used to prepare the PCR amplification mixture) for each batch of samples and the water used to dilute the DNA samples.

The purpose of the negative controls is to detect DNA contamination that might occur from the reagents, the laboratory environment, between the samples being processed, and/or due to improper handling of the samples by the analyst.

The negative controls must be run at the most stringent set of conditions for the batch (e.g., lowest reporting threshold, longest injection time, largest capillary electrophoresis preparation volume). This applies to database samples as well as case work samples.

Verification of the presence of amplified product in the negative controls is performed by viewing the presence of unincorporated primer peaks.

The negative controls should not yield any true STR allelic peaks above the reporting threshold. When peaks greater than or equal to the reporting threshold are present in the range between 80-550 base pairs, the analyst will determine if the peaks are artifacts (e.g. spike, pull-up) or true allele peaks.

The presence of peaks above the reporting threshold will not invalidate the sample as long as the peaks can be shown to be artifacts. Artifacts will be documented (on the electropherogram or the database batch worksheet).

When probable true allele peaks are detected above the reporting threshold, at several or all STR loci, the Technical Manager will be consulted to determine the appropriate course of action.

Since the failure of a negative control may indicate a problem at the extraction or amplification level, the cause of the failure will be fully investigated and documented in the anomaly log book. Steps will be taken (e.g. procedural modifications, corrective action, analyst retraining) to minimize recurrence. Formal corrective action reports may be completed based on the nature of the discrepancy. The Laboratory Quality Assurance Manual and DNA Quality Assurance Manual contain additional information on this subject.

6.3 Internal Control Specimen (ICS)

An internal control specimen (ICS) is a predetermined quality control sample whose expected profile is already known. The ICS is processed with an extraction batch and carried through the batch concurrently with the other samples. The purpose of this

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control is to demonstrate that all analytical processes are working at optimal levels. ICS samples may include any known sample, including those obtained from laboratory staff, where the sample has been previously typed to generate a genetic profile.

At least one ICS sample shall be processed with each casework batch, and will typically be extracted with the known reference samples. Approximately 5% of the samples extracted in a database batch will consist of internal quality control samples. The technical reviewer or CODIS Administrator will verify that the correct STR results are obtained for all internal control samples. An electropherogram of the ICS shall be included in the central log folder (for casework batches).

An ICS with a partial profile or no interpretable profile does not invalidate the batch provided other positive / ICS controls in the batch yield the correct results. The Technical Manager, or other designated individual, shall determine whether or not to approve the ICS when only a partial profile or no profile is obtained. The data quality of other positive control samples in the batch and/or the quality of known /reference samples will be reviewed to determine the approval process.

If an incorrect STR profile is obtained for one or more of the control samples in a casework batch, the analyst and/or technical reviewer will attempt to determine the cause of the discrepancy. Generally, case reports from the analyst and the batch of samples will be suspended until the issue is resolved. The discrepancy will be documented and corrective and future preventive actions may be taken as deemed necessary by the Technical Manager. The Technical Manager, or a designated individual, may approve the issue of unaffected reports on a case-by-case basis. This approval will be documented and maintained with the central log folder pertaining to the batch of cases affected by the discrepancy (using the Corrective Action Report Batch Details form found at the back of this document).

When the discrepancy occurs in a database batch, other profiles in the batch will generally not be entered into CODIS until the issue is resolved. The CODIS Administrator, or a designated individual, may approve the entry of select profiles on a case by case basis.

6.4 Interpretation of Samples

Ideally, allele peak heights should fall between 500 and 2000RFU. Peak heights outside this range may be acceptable; however, it is possible that these samples will require careful interpretation. Alleles with peak heights less than 500RFU may occasionally exhibit peak height imbalance due to stochastic effects caused by low template copy number.

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If one or both Amelogenin peaks fall below 100RFU for a sample, the sample may be analyzed at 50RFU in order to determine the data at the Amelogenin locus. This allowance shall be applied only to the Amelogenin locus. The analyzed data and the relevant electropherogram shall document the lowered threshold for the Amelogenin locus, the peak height(s) and the base pair size(s) of the X and Y peaks. Amelogenin data determined and documented in this manner is suitable for inclusion in the STR results table and entry into CODIS.

6.4.1 General Guidelines

True alleles are peaks that are detected and labeled by the GeneMapper™ ID-X software with a number and should lie within the range of the alleles in the ladder.

Peaks that are labeled as OL (off ladder) at a particular locus have a base size that is different from any of the alleles in the ladder and any of the virtual alleles. These peaks may be true alleles but additional analysis is required to verify this.

Casework

For all casework questioned and reference samples, the minimum reporting (analytical) threshold is 100RFU. Allelic peaks greater than or equal to 100RFU, that are reproducible if re-amplified, may be included in the STR results table and used in statistical analyses. Only alleles equal to or greater than 100RFU will be considered in data interpretation.

A genetic profile will not be considered suitable for comparison when alleles are detected in less than 4 of 12 core STR loci with peaks (homozygous / heterozygous / partial) equal to or greater than 100RFU. This is applicable to both single source samples and DNA mixtures. Exceptions for single source samples may be appropriate. The Technical Manager will be consulted for determining the appropriate course of action for any exceptions to routine guidelines.

When peak(s) below 100RFU exhibit optimal peak morphology and fall in an allele bin, the electropherogram and the STR results table will be marked by an asterisk (*), indicating data below reporting threshold was observed in this sample.

When peaks are observed below the reporting threshold of 100RFU, an additional electropherogram showing a close-up view of the affected loci should be printed for review and inspection. The electropherogram(s) shall also include a note documenting that data below the reporting threshold was/were observed.

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The initial assessment of a questioned sample STR profile should be done without considering the STR profiles of the known/reference samples for the case. Additionally, the analyst should determine whether a sample is a single source sample or a DNA mixture and determine which alleles may be reported, prior to comparing the questioned sample to known/reference samples analyzed.

The probative value of samples will be considered when determining if statistical analysis is appropriate for a genetic profile. Probative value of evidence is assessed based on case information and narrative provided in the Request for Laboratory Services form and/or by communication with the investigator(s).

Results that do not require statistical analysis may include genetic profiles consistent with the individual from whom the sample was taken (i.e. when the origin of the sample is not in question). Genetic profiles consistent with persons whose presence is not probative (for example, DNA consistent with a consensual sex partner in the victim assault kit; items collected from the victim's residence which contain DNA consistent with the victim's DNA; DNA at a crime scene that is consistent with the profiles of persons whose presence at the scene is not unusual and/or does not offer any investigative information) also do not require statistical analysis.

Database

Batches of database samples are uploaded into SDIS by creating an Export Table for CODIS. Prior to creating this table, the analyst must confirm that only eligible profiles are marked for export. This is done by selecting the appropriate Specimen Category while in GeneMapper ID-X. Samples not exported to CODIS are marked "no export".

6.4.2 Off-Ladder (OL) Alleles

The allelic ladder contains the most commonly observed alleles for the STR loci. Alleles which are not assigned a numeric designation in the standard ladder will be designated as off-ladder (OL) alleles.

Before determining whether an OL is a true allele or an artifact, the analyst must consider all other possible causes of OL occurrence.

When a true OL allele lies within the range of alleles for a particular locus, the allele will be reported as a variant of the integer (i.e. X.1, X.2, etc.). The appropriate allele call is determined by simultaneously examining the base sizes for the sample allele peak and the associated allelic ladder peaks in the relevant locus.

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When the OL allele lies either above or below the largest or smallest allele in the ladder, the OL allele will be designated as either greater than (>) or less than (<) the allele from the ladder that is closest in proximity to the OL allele.

Casework

OL alleles considered to be true alleles may require re-amplification to confirm. If the OL allele is observed in multiple samples derived from multiple tissue types or sources with the same STR profile, only one of the samples needs to be re-amplified for verification of the OL allele(s). The DNA Technical Manager must approve reporting an OL allele without re-amplification (e.g. limited amount of sample available for analysis).

An electropherogram will be printed for the sample and the associated allelic ladder for the locus, showing the OL allele(s) in the locus, with the bins and base pair sizes.

Database

For interpretation of database samples, the laboratory maintains a record of known (previously observed) OL alleles. When an OL allele has been previously observed within the laboratory and the possibility of an artifact has been eliminated, the allele may be entered into CODIS without re-amplification. If an OL allele is being observed by the laboratory for the first time, the sample will be re-amplified, prior to CODIS entry, to confirm the genotype. On occasion, re-extraction of the sample may be needed for verification. Once confirmed the OL will be added to the record of previously observed OL alleles.

6.4.3 Low-Level DNA Samples

Casework

Forensic DNA samples often contain low levels of DNA. Low-level DNA samples are samples that exhibit allelic peaks below the reporting threshold. When limited DNA molecules are used to initiate PCR, unequal sampling of the two alleles present from a heterozygous individual may occur due to stochastic effects. The resulting genetic profile may exhibit peak height imbalance, elevated stutter, non-reproducible alleles, etc.

Peak height imbalance owing to low template copy number stochastic effects can often be mitigated by re-amplifying the sample with more input DNA. Alternatively, it may be appropriate to perform a duplicate amplification of these samples to assess reproducibility of the data. All components of a DNA sample mixture may not exhibit reliable reproducibility (of allelic peaks) when there are stochastic effects during PCR. This phenomenon will impact which alleles may be reported and used in data.

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interpretation. Only alleles equal to or greater than 100RFU shall be reported on the STR results table and considered suitable for comparison.

Questioned samples with a quantification value of $<0.05\text{ng}/\mu\text{l}$ and greater than zero (or PCR target template estimate of less than 0.95ng), shall be routinely amplified in duplicate to ascertain reproducibility of alleles above and below 100RFU.

Samples with a quantitation result of zero do not require duplicate amplification.

Questioned samples with a quantification value $>0.05\text{ng}/\mu\text{l}$ will not be routinely amplified in duplicate. Data interpretation and reporting of conclusion(s) will depend on whether the profile indicates a single source or mixed DNA sample. When a sample that was not initially amplified in duplicate yields a DNA mixture profile with the probative fraction being the minor or low-level component and/or contributor, re-amplification may be appropriate to assess allele reproducibility of the minor component and/or contributor.

Note: If a sample is amplified in triplicate – the reproducible alleles in at least two amplifications shall be reported on the STR results table and considered suitable for comparison.

Samples with PCR inhibitors may require re-extraction or additional methods of sample clean-up. The DNA Technical Manager must be consulted prior to using methods not defined in this manual.

Database

Peaks may be observed that are below the reporting threshold of 75RFU. When these peaks exhibit peak morphology similar to allelic peaks and fall within an allele bin, the analyst should consider the possibility of allelic dropout. If allelic dropout is suspected in one or more core STR loci, the analyst should re-amplify and/or re-inject the sample to obtain a complete profile. The sample may have to be re-extracted if these methods fail.

If a partial genetic profile is obtained from a sample the analyst will make the “best attempt” to re-process the sample in order to generate a complete genetic profile. The repeat analysis may include any or all of the following: re-extraction using more sample, maximum template quantity in PCR/maximum template volume, maximum allowable PCR cycling conditions, maximum “prep” or amplified sample loading volume for capillary electrophoresis, and, maximum injection time.

Note: Applicable reagent blank and negative PCR control will be processed under the most stringent conditions).

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If the above “best attempt” also fails to generate a complete genetic profile, a maximum of three core STR loci with elevated stutter and/or stochastic issues (e.g., partial dropout, heterozygous peak height ratio less than 50%) may be permissible in a sample. Samples that do not contain at least partial data at each of the 13 core STR loci are not eligible for upload to NDIS. The allele 10 variant at the D5 locus, which can cause issues with allele heights, is an accepted phenomenon and is not counted towards the maximum three core STR loci with stochastic issues. The presence of the observed variant should be documented on the database batch worksheet. The CODIS administrator will determine the eligibility of such profiles for upload. When only partial data is obtained at one or more PowerPlex® 16 STR loci, it is indicated by selecting “Yes” in the “Partial Profile” field in CODIS. The comments field in CODIS may be used to indicate in which loci the dropout is occurring.

6.4.4 Artifacts

Artifacts are not true alleles. Artifacts can occur in data and much of their nature and origin have been determined and documented.

When the sample exhibits artifacts due to an excess of template DNA in several loci, it may be more appropriate to re-amplify the sample with less template DNA.

Casework

When an artifact is present within the size range of the alleles at a locus and the presence of the artifact interferes with interpretation of the data at that locus, the STR results may be suitable for reporting as long as the affected locus is excluded. Re-analysis of the sample may not be necessary, but this will be determined by reviewer and/or Technical Manager input.

The affected locus may be reported as ‘NR’ (not reported) on the STR results table with a similar corresponding notation on the electropherogram.

Alternatively, the alleles at the affected locus may be reported if the locus is omitted from statistical analysis. This is indicated by adding the notation ‘NS’ (locus/data not used in statistical analysis) at the locus on the electropherogram and on the STR results table, when statistics are issued in the report.

When it is not possible to distinguish between an artifact and a true allele, this is indicated on the STR results table and the electropherogram as Artifact/True allele (A/TA).

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Database

Samples uploaded into CODIS must not contain any labeled artifacts or OL alleles. When artifacts are not pervasive throughout the sample and do not interfere with data interpretation, the sample does not require re-analysis. The analyst may remove the artifact label.

Artifact or OL peaks are either deleted or re-labeled by right clicking on the peak and selecting one of the given options. The analyst should note any changes made to the allele calls in the appropriate column on the database batch worksheet.

When an artifact is present within the size range of the alleles in a core locus and the presence of the artifact interferes with interpretation of the data in that locus, re-analysis of the sample may be required to obtain a profile eligible for NDIS. If the interfering artifact is located in one of the Penta loci, the analyst may choose to enter the profile, omitting all data from the affected Penta locus.

6.4.4.1 Incomplete 'A' nucleotide addition (-A or split peaks)

AmpliTaq Gold™, like many other DNA polymerases, catalyzes the addition of a single nucleotide (predominantly adenosine) to the 3' ends of double-stranded PCR products. This non-template addition results in a PCR product that is one base longer than the actual template, and the PCR product with the extra nucleotide is referred to as the "+A" (base peak) form.

The "-A" form is the peak that represents the correct template length. The "+A" peak will normally be the predominant form, and the "-A" should usually not be detected.

The final step of the STR amplification process is a terminal extension step (60°C for 30 minutes) to promote complete non-template 'A' nucleotide addition.

Failure to attain complete terminal nucleotide addition results in "split peaks", two peaks that are one base apart. Typically, split peaks are the result of an excess of template DNA in the PCR reaction.

Casework

If the labeled "-A" peaks are not pervasive throughout the sample and the sample appears to otherwise be a single source sample, an electropherogram may be printed to document the allele call, base size and peak heights at the affected loci. The OL peaks are labeled as such.

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6.4.4.2 Pull-up

Multi-component analysis is the process that separates the four different fluorescent dye colors into distinct spectral components. The four dyes (Fluorescein, JOE, TMR and CXR) emit their maximum fluorescence at different wavelengths, but there is some overlap of the emission spectra. A spectral calibration is performed for a specific dye set to create a matrix that corrects for the spectral overlap.

Pull-up is the result of incomplete separation of the emission spectra and is typically observed as a non-allelic peak at the same base size as a peak in another dye. This typically occurs at high RFU values, when an excess of template DNA has been amplified.

Pull-up may also occur when the matrix file is not current. A new spectral calibration is performed whenever a new capillary array is placed on the instrument or whenever any part of the optics have been realigned and/ or replaced. A new spectral calibration may also need to be performed when an overall decrease in spectral separation is observed.

If the pull-up is prevalent enough to interfere with data interpretation, the sample may need to be re-injected or re-amplified with less DNA template.

Casework

Pull-up that gets labeled as a true allele or OL should be documented by an electropherogram showing the base size for the true allele and the pull-up peak.

6.4.4.3 Stutter

The PCR amplification of tetra-nucleotide STR loci typically produces a minor product peak four bases shorter, or two bases shorter (infrequently observed), than the corresponding base peak. This is the result of slippage of the Taq polymerase and is referred to as the stutter peak or stutter product. In the penta-nucleotide loci, the stutter peak is five bases shorter than the corresponding base peak.

The proportion of stutter product relative to the main allele peak is calculated by dividing the height of the stutter peak by the height of the main allele peak. The stutter percentage is fairly reproducible for a particular locus. The following table lists the values of the expected percentage of stutter for the loci in the PowerPlex® 16 System when run on the Applied Biosystems 3130xl. The stutter values are based on data obtained by internal validation studies as well as other published data (Krenke, B., et al: Validation of a 16-locus Fluorescent Multiplex System. July 2002, J Forensic Sci.,47(4)).

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Maximum Expected Stutter Percentage for PowerPlex® 16 loci on the 3130xl (in single source samples)

D3S1358 13%	TH01 5%	D21S11 15%	D18S51 13%	Penta E 7%	
D5S818 13%	D13S317 10%	D7S820 10%	D16S539 10%	CSF1PO 10%	Penta D 5%
Amelogenin NA	vWA 13%	D8S1179 10%	TPOX 5%	FGA 13%	

Peaks in the stutter position greater than the listed values may indicate the presence of DNA from more than one individual.

As with other artifacts, if the presence of the stutter peaks interferes with data interpretation, the sample may need to be re-injected or re-amplified with less template DNA.

Casework

When there is no indication of a mixture other than elevated stutter, the analyst will document the stutter as such on the electropherogram.

When the sample is a DNA mixture, it may not be possible to distinguish between a stutter peak(s) and a minor component allele(s). In such situations, the peak may be labeled as an Artifact/True allele (A/TA). This shall be documented on the electropherogram (and on the STR results table if appropriate).

Database

The GeneMapper™ ID-X database analysis methods have a global stutter filter of 20%. Peaks in the stutter position greater than 20% may indicate the presence of DNA from more than one individual. When there is no indication of a mixture, and the data is determined to be elevated stutter, the analyst will document the elevated stutter on the database batch worksheet. It is unlikely that the presence of stutter peaks would interfere with the interpretation of database samples and therefore, re-amplification and/or re-injection of the sample may not be required.

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6.4.4.4 Spikes

Spikes are non-allele peaks that may arise due to air bubbles, urea crystals or voltage spikes. Spikes are typically quite sharp and easy to distinguish from a true allele.

Spikes usually appear in more than one color at the same base size and are not reproducible by re-injection.

If the presence of a spike(s) interferes with the interpretation of the sample, the sample will be re-injected.

Casework

If the sample does not require re-injection, the spike(s) shall be documented as such on the electropherogram.

Database

Peaks labeled as such by the GeneMapper™ ID software cannot be manually removed. However, they do not interfere with upload to CODIS and no re-analysis is required if the spike does not interfere with interpretation of the sample.

6.4.4.5 Dye Blobs

Dye blobs, like spikes, are non-allele peaks that are easily distinguished from true alleles. Dye blobs may be the result from residual dye molecules left over from the synthesis of the primers or they may be dye molecules that fell off the primer during the amplification.

Samples with dye blobs that interfere with data interpretation will be re-injected.

Casework

If the sample does not require re-injection, the dye blob(s) shall be documented on the electropherogram as artifacts.

6.4.4.6 Persistent Kit Artifacts

Occasionally, one or more lots of PCR kits may exhibit persistent artifacts that may not be observed in the verification process. These artifacts may or may not appear as true alleles. Sometimes, these artifacts are so common that they are observed in a high number of routine samples.

If the presence of the kit artifact does not interfere with data interpretation, the Technical Manager shall determine if the artifact can be documented as such, without requiring re-analysis of the sample.

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When the Technical Manager has acknowledged a particular kit artifact based on verification of the kit lot, his/her documented approval is not required on subsequent electropherograms containing that artifact. This acknowledgement will be documented and retained with the kit verification paperwork.

6.4.5 Single Source Samples

Typically, each locus is characterized by one or two labeled peaks or alleles. If two peaks/alleles (heterozygous locus) are detected at a locus, they should ideally be of equal intensity – that is, the peak height ratio (PHR) is approximately 1:1.

Peak height ratios are calculated by dividing the peak height (in RFUs) of the lower RFU allele by the peak height of the higher RFU allele, and then multiplying this dividend by 100 to express the PHR as a percentage. The minimum expected PHR for single-source samples, where there is no indication of a mixture, is flagged in GeneMapper™ ID-X at 60%. This ratio, however, may be lower with lower amounts of DNA. Peak-height-ratio imbalance may also result from degraded DNA or the presence of PCR inhibitors. Allelic dropout may occur in degraded/inhibited samples and the possibility of a second allele should be considered.

Samples with more than two peaks at a locus or with alleles of significantly different peak heights may indicate the presence of mixtures, tri-alleles, primer binding site mutations/variants or other biological or procedural artifacts. Such observations require careful consideration.

Casework

The frequency of a single source profile (including single source major/minor components from a DNA mixture) will typically be calculated when STR results cannot exclude an individual as the source of DNA in the sample.

The probative nature of single source matches will be considered when determining if statistical analysis is appropriate. It is not necessary to calculate a profile frequency when the questioned sample is an intimate sample (i.e., body swabs) and there is no DNA detected that is foreign to the “owner” of the sample (i.e. the person from whom the sample was collected) or if the questioned sample is consistent with a consensual and/or elimination known/reference sample(s).

Loci where the reported genotypes are identical between the questioned and the known sample shall be used in the statistical analysis and the frequencies of such profiles reported.

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6.4.5.1 Tri-alleles

Occasionally, a single-source database sample may be observed to have three alleles at one (rarely more) locus/loci. Tri-allelic patterns have been observed in single source samples and there are documented instances where different tissues from the same individual may or may not exhibit the tri-allelic pattern.

Confirmed tri-alleles may be entered into CODIS, in eligible offender and forensic unknown specimens.

Casework

Samples exhibiting potential tri-alleles may be re-extracted and re-amplified to confirm the genotype observed at the locus (or loci). Statistical calculations, however, will not be performed at these loci.

If the potential tri-allele containing sample is re-extracted and re-amplified and found to be reproducible, the analyst may include the locus with the tri-allele in the STR results table. If the potential tri-allele is not reproducible, it will be reported as an Artifact/True allele (A/TA).

If the same tri-allelic pattern is also observed in multiple samples (from the same case) but from different tissue sources – for example, blood / semen / saliva / hair, with the same STR profile, the potential tri-allele may be considered a reproducible observation and may be included on the STR results table without the A/TA designation. No re-extraction of samples would be required in such instances.

Database

Samples exhibiting tri-alleles will be re-amplified and may have to be re-extracted to confirm the observed genotypes.

6.4.5.2 Comparison of DNA Results

The interpretation process generally results in one or more of the following conclusions:

- Exclusion
- Cannot exclude
- Inconclusive
- Insufficient DNA/Data
- No genetic profile

Exclusion (Non-match):

An exclusion is declared when the known sample has alleles that are not observed in the questioned sample and there is no scientific explanation for the non-match.

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Scientific explanations may include, but are not limited to, factors such as inhibition, degradation, low template DNA and/or primer binding site variations or mutations.

Parent-offspring exclusion (also Parent-offspring inconclusive) is declared when, upon comparison of the DNA results from an offspring sample to that from an alleged biological parent(s), the profiles are found to not share at least one allele at two or more corresponding loci.

Cannot exclude (Match):

The donor of the known sample cannot be excluded as a source of the questioned sample when there are no significant differences between the allele designations obtained from these samples. The Random Match Probability (RMP) will be calculated for the forensic sample profile.

Partial Profile: If one allele is identified and a second potential allele is below the reporting threshold (100RFU), the identified allele will be used for comparison purposes first and if concordance is established the peak below threshold will be assessed to support the inclusion and/or exclusion of an individual(s). Any locus/loci that is utilized for an inclusion based on the individual's allele(s) falling in a peak below threshold position will not be used in the statistical calculation. The significance of a match will decrease for partial profiles because there are fewer loci to compare.

Parent-offspring inclusion is declared when, upon comparison of the profiles obtained from an alleged offspring sample and that of an alleged biological parent(s), at least one allele is shared at each locus for which interpretable DNA results were obtained.

Inconclusive:

When the nature of a forensic genetic profile is such that a meaningful comparison to a known sample genetic profile and/or another forensic genetic profile, cannot be made due to varying reasons, then the results are deemed inconclusive. An 'inconclusive' can be reported in regards to whether a particular person can/cannot be excluded as a source of DNA in a particular sample. The same data (electropherogram) may however, if appropriate, be used to draw conclusions about other case related individuals' potential DNA contribution(s) to the sample.

Insufficient DNA/Data:

If insufficient DNA is observed in a sample (e.g. data below reporting threshold, drop-out, low-level DNA) the data may not be suitable for comparison. This data will be considered insufficient and cannot be utilized for meaningful comparative analysis. As such, this data will be omitted from the STR results table.

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No genetic profile:

No genetic profile is declared when there is no data observed. This also may include low-level data observed at less than 4 of 13 core STR loci.

6.4.6 DNA Mixtures

DNA mixtures derived from more than one individual are frequently identified in biological evidence recovered in forensic investigations. DNA mixtures may consist of blood, semen, saliva, epithelial cells or a combination of any of these sources. Such evidence may be derived from:

- intimate swabs/samples collected from an individual
- blood/contact DNA found on weapons used in assaults or homicides
- wear areas on clothing
- biological evidence recovered from high traffic areas (i.e., floors, carpets, door handles, steering wheels, etc.)
- other biological samples collected from crime scenes

An evidence item taken directly from an identified anatomical location (i.e., vaginal swab, penile swab, fingernail scrapings, etc.) and / or a piece of intimate apparel (i.e., undershirts, panties, bra, etc.) typically will yield DNA from the individual from which the evidence item was taken. In such circumstances, it is common to recover a DNA mixture which includes DNA consistent with that individual. It may be possible to deduce the unknown contributor, and issue a single source statistic, using the "owner's" reference sample.

All loci for which DNA results were obtained must be considered when evaluating potential mixed samples. A sample may be considered to have originated from more than one individual when more than two alleles are observed at more than two loci. The minimum number of contributors may be defined by evaluating the locus that exhibits the most allelic peaks (i.e., if at most five alleles are detected at a locus, the DNA results are consistent with having arisen from at least three individuals).

Identification of DNA mixtures can be straightforward or challenging, depending on the number of loci typed, the number of contributors, the total amount of DNA recovered, and the relative proportion of DNA/contributed by each individual to the mixture. Therefore, interpretation of DNA mixtures and statistical analyses requires careful consideration of factors affecting allele designation, heterozygote detection and peak height variations within and between loci.

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Indications of a DNA mixture sample can include:

- (1) more than two alleles at two or more loci,
- (2) a peak in a stutter position that is unusually high
- (3) significant peak height ratio imbalance for a heterozygous genotype
- (4) Observation of low-level DNA/data below reporting threshold

Occasionally, factors other than DNA mixtures may influence such observations. Before determining that a sample is a mixture, the analyst shall eliminate the possibility of split peaks, pull-up, stutter, kit artifacts, and low template stochastic effects. When a sample is determined to be a mixture, the analyst must consider whether it can be dissected into major and minor components. The number of potential contributors of DNA and intra-locus peak height ratios should be considered when making these determinations. As the number of potential contributing sources in a mixture increases or, if the number of shared alleles between the contributing sources increases, dissecting a mixture profile into major and minor components becomes increasingly challenging and should be approached with caution, if at all attempted.

Major component allele peaks, even at several loci, do not necessarily indicate the presence of a single major contributor. Depending on the number of DNA contributors and the degree of allele sharing, the major component may be comprised of DNA from more than one individual. Similarly, alleles designated as minor component alleles often may not reflect the entire genotype of a minor contributor owing to allele dropout or allele sharing.

An analyst may not dissect a mixture profile into major and minor components if a mixed sample profile indicates the possibility of several contributors or many (potentially) shared alleles between the contributors.

Caution must be exercised when dissecting a mixture into major and minor components. Detailed notes should be made on the electropherogram to document the rationale behind the interpretation.

Input from the DNA Technical Manager may be required to resolve analysis and interpretation of such data. Approval, if required, should be documented by the Technical Manager's initials and date on the electropherogram.

The probative nature of DNA mixtures will be considered when determining if statistical analysis is appropriate. It is not necessary to calculate a profile frequency when the questioned sample is an intimate sample (i.e., body swabs) and there is no DNA

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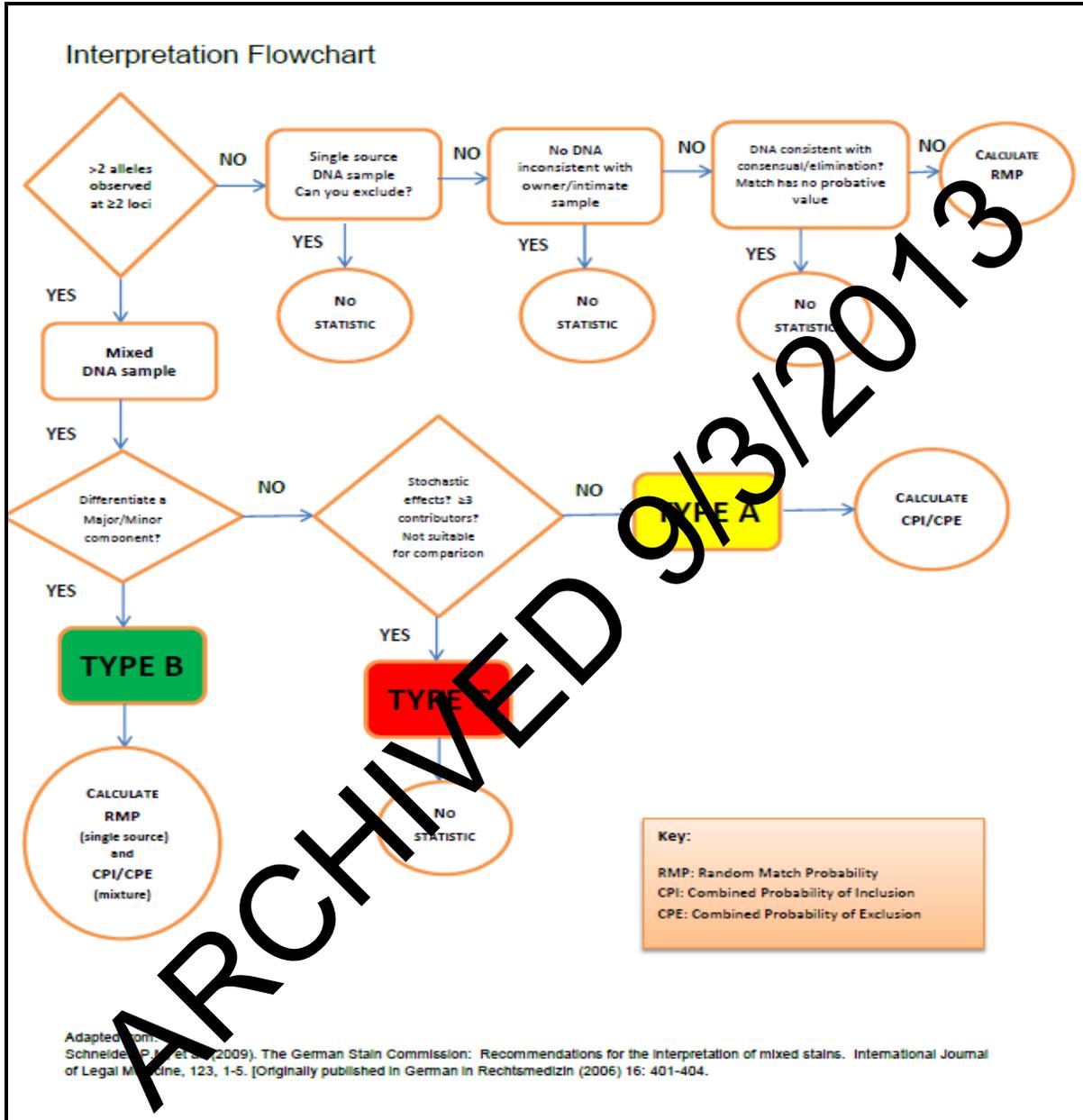
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detected that is foreign to the “owner” of the sample (i.e. the person from whom the sample was collected) and a consensual and/or elimination known/reference sample(s).

When issuing statistics on a mixture profile, only the loci where all alleles are reproducible (when the sample is amplified in duplicate) and where no allele dropout is observed, will be used in calculating the statistics.

When data suggests that the major/minor component allelic peaks are consistent with being from a major/minor contributor (i.e., it is possible to deduce a major/minor single source from the mixture), a single source statistic (profile frequency) may be issued in addition to the mixture statistic (probability).

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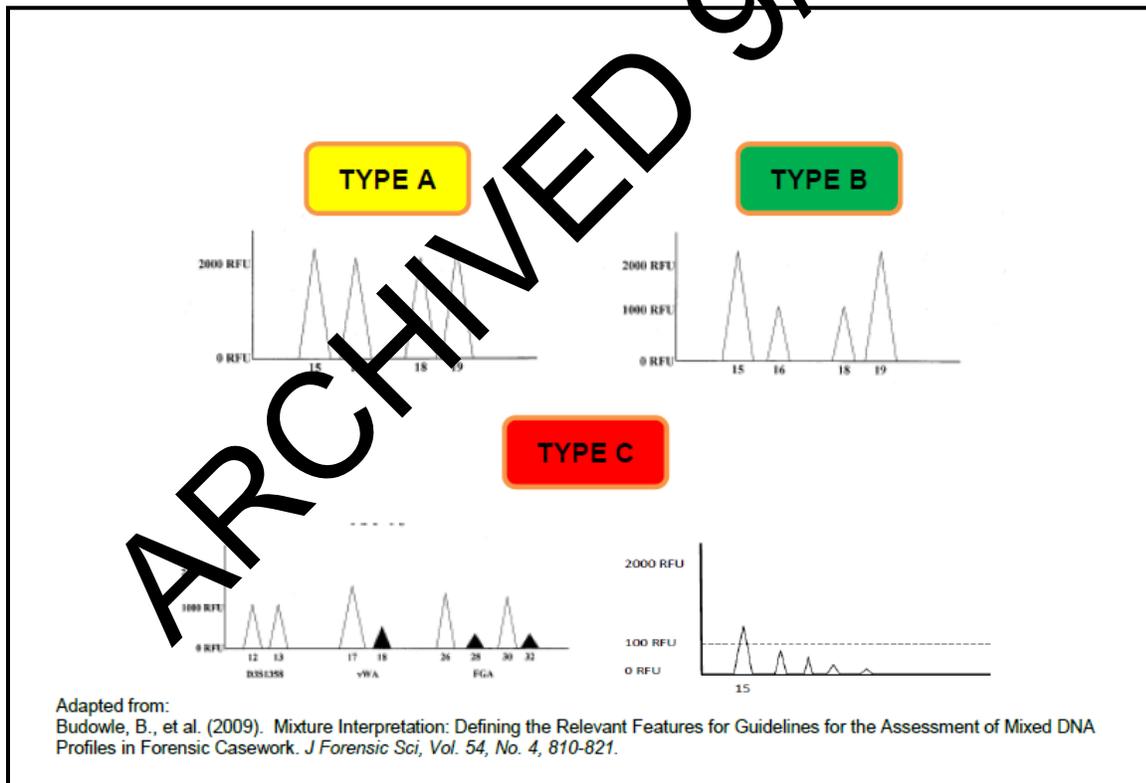
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6.4.6.1 DNA Mixture Type Categorization

It is not possible to anticipate the nature of all potential DNA results, or the nature of the evidentiary samples from which they may be obtained. These categories do not exhaust the possibilities that may be encountered by the forensic scientist, nor the conclusions that may be rendered from his/her interpretation of the results.

Three categories of mixed samples have been designated to guide general decision making as illustrated in the Interpretation Flowchart. Mixtures with a known contributor (i.e. intimate samples) are possible in each category:

- **TYPE A:** mixtures with indistinguishable contributors, including multiple major contributors
- **TYPE B:** mixtures with major/minor contributors
- **TYPE C:** mixtures containing DNA from at least three sources and/or mixtures that exhibit potential stochastic effects (e.g. 1 major plus 2 or more minor contributors, 2 major plus 1 or minor contributor indistinguishable)



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TYPE A:

Description: If the amounts of biological material from multiple contributors are similar, it may not be possible to further refine the mixture profile. When major or minor contributors cannot be distinguished because of similarity in signal intensities (i.e. peak heights), the sample is considered to be an indistinguishable mixture.

The classification of indistinguishable does not imply that the profile is not interpretable.

Individuals may still be included or excluded as possible contributors to an indistinguishable mixture. When appropriate, statistical analysis may be performed using the mixture formula.

TYPE B:

Description: If the amounts of biological material from multiple contributors are dissimilar, it may be possible to further refine the mixture profile. When major or minor contributors can be distinguished because of differences in signal intensities (i.e. peak heights), the sample is considered to be a distinguishable mixture. The difference is evaluated on a case-by-case basis.

Separating a two-source mixture into major and minor components is easier when the number of loci exhibiting four alleles increases and the ratio of the major contributor to the minor contributor increases above a 2:1 ratio.

If a mixture can be successfully separated into major and minor components, the minor component alleles are reported within parentheses. Due to the possibility that the minor contributor's alleles may be shared by the major contributor and that such alleles may be below reporting threshold, determination of the minor contributor profile may be possible at only some loci.

The minor component may be suitable for comparison if alleles equal to or greater than 100RFU occur at least 4 of 13 core loci. When evaluating if a minor component is suitable for comparison the number of potential contributors and whether the peak falls into stutter position should be considered.

An analyst may partially dissect a DNA mixture profile into major and minor components and report the loci on the STR results table in brackets (e.g. [10,11,12]) where an unambiguous major or minor component is not distinguishable. Peak height ratios may be noted on the electropherogram to document possible genotypes, if appropriate.

Distinguishable mixtures may be used for the purposes of comparison. A Random Match Probability statistic may be calculated and reported for single source major

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and/or minor component(s). The mixture formula may be used when an individual cannot be excluded from the sample when considering all reportable (equal to or greater than 100RFU) alleles.

TYPE C:

Description: In general, type C mixtures contain DNA from at least three (3) individuals and may also exhibit stochastic effects.

If the sample demonstrates a clear reproducible major component at a minimum of 10 of 13 core STR loci a conclusion as to the source of the major contributor DNA may be reported and will be included in statistical analysis. This may also encompass mixture profiles that exhibit more than one major contributor.

If no clear major component is observed this type of genetic profile may be deemed unsuitable for comparison. As such, this profile will not be included on the STR results table and no statistical analysis will be performed.

A sample may be deemed not suitable for comparison if the majority of alleles in a sample are not reproducible and/or insufficient DNA is detected (i.e. partial profile). Additionally, if a mixture contains DNA from known close relatives a comparison may not be appropriate.

6.4.6.2 Comparison of DNA Results

The interpretation process generally results in one or more of the following conclusions:

- Exclusion
- Cannot exclude
- Inconclusive
- Insufficient DNA/Data
- Complex mixture

Exclusion:

An exclusion is declared when the known sample has alleles that are not observed in the questioned sample, and there is no scientific explanation for the absence.

Cannot Exclude:

When alleles detected in a known/reference sample are also detected in at least 10 of the 13 core STR loci in a DNA mixture, and when there is a scientific explanation for any inconsistencies, an individual may be considered a potential contributor to the sample.

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If alleles that could not have been contributed by the individuals for whom known/reference sample profiles were submitted for comparison with the questioned samples, the conclusions for this sample may include a statement indicating that DNA that could not have been contributed by X/Y/Z was detected in the sample.

Partial Profile: If reportable alleles (100RFU or greater) are detected in a mixture at less than 10 core loci the sample may still be suitable for comparison. The Technical Manager will be consulted to determine the appropriate course of action.

Note: If a genetic profile obtained from a sample does not satisfy the laboratory's inclusionary reporting criteria it is possible it may be utilized for exclusionary purposes only. The Technical Manager will be consulted to determine the appropriate course of action.

Inconclusive:

If the STR results neither support an inclusion (i.e. DNA consistent in at least 10 of 13 core STR loci) nor an exclusion, the comparison will be deemed inconclusive and reported as such.

Insufficient DNA/Data:

If insufficient DNA is observed in a sample (i.e. data below reporting threshold, drop-out, low-level DNA) the data may not be suitable for comparison. This data will be considered insufficient and will not be utilized for comparison. As such, this data will not be included on the STR results table.

Complex Mixture:

A complex mixture may be declared when DNA from multiple sources is detected in a sample, there is evidence of stochastic effects (i.e. drop out) at several or all loci (from limited DNA), a clear major component cannot be deduced, and/or a majority of the alleles are not reproducible when amplified in duplicate. Accordingly, the sample will be deemed not suitable for comparison.

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6.4.7 Consensus/Composite Data Reporting

Casework

When a sample is amplified in duplicate, only the reproducible alleles are generally included on the STR results table.

On occasion, reporting data composited from multiple injections/amplifications (e.g. data from more than 2 amplifications) from the same extract may be deemed appropriate after inspection of the quality of data. Such data will require documented technical manager approval for compositing.

Database

Data will not be composited from multiple amplification/injections of a database sample to obtain a more complete profile.

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Section 7 Case Records Documentation and Report Writing

Refer to the Laboratory Quality Assurance Manual for general laboratory guidelines governing casework documentation and report writing, and for information regarding discovery requests and the release of information from the laboratory.

The following abbreviations are commonly used in bench notes and on other documents generated during analysis.

Biological Screening Abbreviations and Definitions	
Phenolphthalein/Kastle-Meyer	PH, Pheno. of KM
Fluorescence/Alternate Light Source	F or ALS
Acid Phosphatase activity	AP, BCIP, STMP
Nucleated Epithelial Cells	NECs
Prostate-Specific Antigen	PSA or p30
Human Hemoglobin	hHb, Species
Positive test result	Pos or (+)
Negative test result	Neg or (=)
CIDI	Case,item,date & initials

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DNA Abbreviations and Definitions	
BL	Noisy baseline
SH	-A / +A Shoulders
PHR	Peak Height Ratio <60%
SPK	Spike
PU	Pull-Up
DO	Complete Drop-Out at a Locus
PDO	Partial Drop Out at a Locus
D5Mut	D5 Mutation
OL	Off Ladder
OMR	Outside Marker Range
OS	Off Scale/Saturation
NP	No genetic profile obtained
NR	Data not reported
NS	Data not used for statistics
A/TA	Artifact (i.e. stutter) or true allele
IF	Instrument failure
ILS	ILS failure
DBRT	Data below reporting threshold
ART	Artifact
TRI	Tri-allele
()	Minor component allele
[]	Major/Minor components not separated
LV	Large Volume (EZ1 protocol)
RBQ (-LV)	Questioned reagent blank, direct extraction
RBE/RBS (-LV)	Epithelial and sperm reagent blanks
RBK	Known reagent blank
CIDI	Case,item,date & initials

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7.1 Documentation Generated During Screening and DNA Analysis

All paperwork generated during the course of analysis will be maintained either in the case record, or in a central log record in the LIMS or on the laboratory network share drive.

All pages in the case record and central log record contain the case number, analyst's initials, item # (if appropriate, i.e. digital images) and page number (page X). The total number of pages is indicated by marking the last page as "last page".

When one analyst performs an analysis on behalf of another analyst, the resulting data pages should contain the handwritten initials (or secure electronic equivalent) of each analyst.

7.1.1 Biological Screening Bench Notes

Biological screening bench notes consist of any documentation generated during the analysis of a case and are specific to that case. The biological screening bench notes are placed in the case images for the request in the LIMS. All bench notes must be in LIMS and the report marked draft complete (by the analyst) prior to submitting the case for technical review. Upon completion of the technical and administrative reviews, the review checklist is also added to the case images.

The bench notes for each case may contain the following worksheets:

- Victim Assault Kit Worksheet
- Suspect Assault Kit Worksheet
- Evidence Examination Worksheet
- Microscopic Worksheet
- PSA/Semen Identification Worksheet
- HemaTrace®/Species Identification Worksheet
- Case Images Worksheet

The worksheets contain details of all of the items processed and include the item packaging, contents and description, images of the evidence processed (when applicable), documentation of all presumptive tests performed and the test results, the location of all testing, the location of all isolated stains/samples, trace evidence collected (if applicable), the reagents used, and the date the evidence was opened and sealed (where applicable) in accordance with laboratory policies and procedures outlined in the Laboratory QA Manual.

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7.1.2 DNA Central Log Records

Each batch of cases assigned to a DNA analyst will be named with the batch date (typically, this is the day you take custody of the evidence) and the analyst's initials (i.e. YY-MMDDinitials 06-0102MLC). This batch name is recorded for each case in the LIMS. Upon completion of the technical review of a batch, the central log documentation is retained in LIMS, in both the case record and the annual DNA record.

The templates for the worksheets that comprise the central log are controlled documents. The templates may be modified as required for the batch. The central log for each batch of cases will contain at least the following information in an appropriate format:

- A cover sheet containing:
 - batch name
 - the name of the analyst(s)
 - a list of cases worked in the batch
 - the raw data folders and GeneMapper M (current version) projects for all data used to generate results/conclusions for cases in the batch.
- A Casework Standards, Controls and Reagents worksheet detailing:
 - the extraction methods used
 - specific instrument used
 - elution volumes
 - the extraction controls
 - date of reagent blank (should be on or after the date of extraction)
 - extraction reagent lot numbers and reagent expiration dates. (reagents not used can be deleted from worksheet)
- Q-PCR worksheet(s) containing the lot numbers and expiration dates of reagents/standards used in quantitation as well as a map of the 96 well plate.
- The standard curve for the quantification.
- The quantitation results sheet (Initial template quantity) showing the estimated concentration of all samples quantified (handwritten, avg of the 2 values obtained for Q's).
- The amplification/electrophoresis worksheet(s) detailing:
 - the samples/controls amplified (identify clearly the Positive Control sample provided by the PCR kit vendor).
 - the well plate locations
 - the amount of sample amplified
 - the amount of sample (amplified product) injected for electrophoresis,
 - the electrophoresis injection modules,
 - the lot numbers and expiration dates of all reagents used in amplification and electrophoresis.

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- If electrophoresis results indicate that a sample should be re-extracted, re-amplified or re-injected, the reason should be documented in the comments/notes field of the amp/3130 worksheet (See table above for list of abbreviations)
 - Instrument failure
 - ILS failure
 - Noisy baseline
 - Data below reporting threshold
 - Drop-out
 - Saturation
 - Off-ladders
 - Artifacts (ie pull-up, dye blobs, spike, bubbles, shoulder)
 - Potential Tri-allele
 - Data above reporting threshold in blank/negative control

Note: Multiple injections of the same sample at more than one injection time (for efficiency purposes) in a CE run do not require documentation of a reason. The analyst will select the injection best suited for interpretation, based on the interpretation guidelines

- Electropherograms for all control samples generated during analysis of the batch.
 - ILS - The printouts must show the correct labeling of the 80-550 peaks of the internal lane standard and the tops of every peak.
 - Negative/blank controls - primer peaks must be visible by selecting Blank Casework as the Analysis Method. The negative/blank controls must be injected for at least as long as the longest injection time of a casework sample. [Plot setting: Casework Blank]
 - ICS - print and label ICS and add to central log as a control. The analyst should include the necessary information for the reviewer to verify that the obtained profile matches the expected profile. This additional documentation is not retained once the technical review is completed.
 - Root/shaft hair extraction internal controls
 - Exceptions must be approved, in writing, by the Technical Manager or another designated individual.
- Electropherograms of all allelic ladders used for genotyping (Print in landscape view (horizontal) with ILS).
- Page numbering of the central log does not need to be completed until all laboratory work is completed for all cases in the batch.

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7.1.3 DNA Bench Notes

DNA bench notes consist of any documentation generated during the analysis of a case that is specific to that case. Upon completion of a case, the DNA bench notes and the DNA laboratory report are maintained in the LIMS. The templates for the DNA worksheets that comprise the case record are controlled documents. The templates may be modified as required for the case. The bench notes for each case will contain at least the following:

- The STR Results table (an administrative document) comprises the first page(s) of the bench notes and contains genetic profiles from questioned and known samples suitable for comparison.
 - It is not necessary to include in the STR Results table the profiles obtained from samples that serve as internal controls (i.e. the epithelial fraction of a vaginal swab) as long as the profile is consistent with expected results
 - If results are not suitable for comparison, do not include in the STR Results Table the Questioned sample profile (i.e. sample with no called alleles, majority of alleles are not reproducible, minor profile not suitable for comparison, etc).
 - If interpretable genetic profiles are not obtained from any of the questioned samples, the known sample profiles need not be included in the STR Results Table.
 - Add a * after the item description if there is data – i.e. alleles - below reporting threshold in a sample.
 - Add “epithelial” or “sperm fraction” to sample description when necessary.
 - Minor alleles go in () below the major alleles.
 - Add “major component” in the item description when only reporting the major component of a mixture.
 - If an allele is determined to be an A/TA, this notation goes after the allele call in superscript (i.e. 10^{A/TA}).
 - Loci with no reportable alleles are left blank and the boxes grayed out.
 - All notes at the bottom of the STR Results Table are retained.

Note: For Proficiency Tests, it is not necessary to create a separate a STR Results table. The DNA results and conclusions pages of the paperwork from the test provider are completed and become the first pages of the DNA bench notes.

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- The DNA extraction worksheet(s) contains:
 - All of the questioned and known items processed,
 - Sample code (within the batch)
 - Documentation of item packaging
 - Presumptive testing performed and the results if applicable
 - Amount of sample used (sample size)
 - Date sampled (this is the date that you cut your evidence)
 - Extraction methods and specific instrument used
 - Elution volumes
 - Quantitation results
 - Disposition of the DNA extract
- Digital image printouts of questioned samples, when appropriate (ie cigarette butts, fingernail clippings, hairs).
- Electropherograms for all of the items amplified, showing the correct labeling of the 80-550 peaks of the internal lane standard [Plot setting: Traditional Genotype Plot]. All electropherograms must contain the job # and item # in the Sample Name column.
 - If the sample has no labeled peaks, the electropherogram must also include the primer peaks [Analysis method: Blank casework; Plot setting: Casework Blank]. Exceptions must be approved, in writing, by the Technical Manager or another designated individual.
 - Zoomed in views (electropherograms) for samples may be appropriate when there are artifacts [Plot setting: Casework artifact] . Zoom in on locus with artifact, there is no need to print entire profile. For data below the reporting threshold [Plot setting: Casework zoom]- Print entire profile.
 - If analyzing at 50fru for Amelogenin, print a second electropherogram zoomed [Plot setting: Casework artifact] for Amel locus only. Add note "Analyzed at 50fru for Amel only" to main electropherogram and hand write in a "Y" and circle it.
 - Special case: When an OL is a true allele or a potential tri-allele exists, print using Traditional Genotype Plot. Also print a casework artifact view that includes the allelic ladder and the locus on one sheet.
- The following handwritten notations may be included on the electropherogram:
 - "DBRT" in the upper right hand corner
 - Loci with peak height ratio <60% in a single-source sample (highlighted in orange in GMID-X) must be initialed
 - Artifacts (i.e. pull-up, dye blobs, etc.) are noted as "artifacts" on both the traditional genotype view and the zoomed in electropherogram
 - "No genetic profile" added to bottom when no reproducible alleles are above reporting threshold

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- For samples that have been double amplified and yielded data suitable for comparison, add “Reproducible alleles (included) on STR Results Table” to bottom of the first electropherogram. All non-reproducible alleles are crossed out and initialed on all electropherograms.
 - For samples that were double amplified and did not yield data suitable for comparison, add “Reproducible alleles noted” and the reason that the data is not suitable for comparison to the bottom of the electropherogram
 - For peaks in stutter positions (that appear close to the expected stutter percentages) indicate the % of the peak relative to the main peak and the expected stutter percentage (for example, 12%>5% or 5.2%~5%). When the peak can be reasonably interpreted as elevated stutter, add a notation to this effect. When it is not possible to discern whether the peak is a stutter peak or a true allele, add “A/TA”.
 - If data is not suitable for comparison, add a note at bottom of electropherogram i.e. “data not included in STR Results table due to insufficient DNA”, “minor component not suitable for comparison therefore not included in STR Results table”, “minor component consistent with owner and therefore not included in STR Results table” etc.
 - If able to determine a major vs. minor profile add () around the minor alleles.
 - ‘NS’ at loci determined not to be suitable for inclusion in a statistical analysis
- Popstats printouts for all samples for which a statistical analysis was performed will include:
 - Specimen ID: Lab#_Item#
 - Comments section: add any additional info (ie sperm fraction, major profile)
 - Print for all populations
 - The casework review checklist, completed and signed by the analyst and reviewer
 - Analyst will complete the first column of boxes before handing in for technical review

Note: All pages in file must be page numbered before handing in for technical review.

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7.2 Report Writing

Proficiency test reports will be written in a manner similar to casework reports. Proficiency tests for DNA reports will not require the STR results table used for casework reports. Instead, the completed STR table from the test provider's form will be included as the first page in the bench notes.

7.2.1 Guidelines for Biological Screening Examinations / Reports

All biological screening reports will contain the following:

- Date report was issued
- Laboratory and agency case numbers (automatically populates)
- Name of submitting agency and case officer (automatically populates)
- List of all items analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #
- Results of presumptive/confirmatory tests (in body of report)
- Results, conclusions and opinions for all tested items (guidelines for reporting are provided in the tables below and may be modified, as necessary, on a case by case basis)
- Explanation of why testing was stopped, if appropriate
- The disposition of all retained items
- Any known samples that are required for DNA analysis, when applicable
- Statement regarding STR testing, when applicable
- Signatures of the reporting analyst and the technical reviewer (the analyst should electronically sign the report by setting the milestone to Draft Complete prior to submitting for technical review; only the analyst is required to scan their barcode and enter a pin when signing the report in LIMS)

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Sperm Findings	Report
Microscopic examination for spermatozoa	Spermatozoa/few spermatozoa/no spermatozoa were observed/detected (microscopically) in/on ...
Blood Findings	Report
Phenolphthalein (Kastle-Meyer)	<p>A stain(s) testing positive/negative with a presumptive test for blood was/were located/detected on (item).</p> <p>No blood was detected on (item) with a presumptive test for blood.</p> <p>This stain/sample tested positive/negative to a presumptive test for blood.</p>

Seminal Fluid	Report
BCIP/STMP negative	No semen was detected chemically (BCIP/STMP) on (item)
BCIP/STMP positive	Positive results are not reported
P-30/ABA card positive (no spermatozoa observed)	<p>The presence of PSA/semen was detected on (item) by immunoassay. This test detects the presence of the human prostate specific antigen (p30) found in seminal fluid.</p> <p>Item (#) has been retained in the laboratory and may be suitable for Y-STR analysis. For more information please contact the laboratory's DNA Technical Manager, Abirami Chidambaram (269-5621) or (abirami.chidambaram@alaska.gov).</p>
P-30/ABA card positive (no spermatozoa observed)	No PSA/ semen was detected on (item) by immunoassay.

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Speciation	Report
ABA card positive	Stain(s) present on (item) tested positive using an immunoassay test for the presence of human hemoglobin. This test is specific to human, higher primate and ferret blood.
ABA card negative	No human hemoglobin was detected by immunoassay.

Hair/Fiber Evidence	Report
Hairs/Fibers recovered (positive biological findings)	Hairs/fibers were observed/recovered from (item).
No Hairs/Fibers recovered	No human hairs were observed/recovered in/from
Human hairs (suitable for nuclear DNA)	A human head hair(s) was/were found on/in (item). Tissue or tissue-like debris was observed stereoscopically on the root(s). This/These hair(s) may be suitable for nuclear DNA.
Human hairs (not suitable for nuclear DNA)	A human head hair(s) was/were found on/in (item). No tissue or tissue-like debris was observed stereoscopically on the root(s). This/These hair(s) are not suitable for nuclear DNA. No further examinations were conducted.
Human hair fragments (not suitable for nuclear DNA)	One hair fragment, not suitable for nuclear DNA analysis was found in/on (item/envelope). No further examinations were conducted.

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7.2.2 Reporting Guidelines for DNA Analyses

All DNA discipline reports will contain the following:

- Date report was issued
- Laboratory and agency case numbers (automatically populates)
- Name of submitting agency and case officer (automatically populates)
- List of all items analyzed
 - include the agency item # in () if the item was re-numbered at the laboratory
 - this is not necessary if the laboratory number contains the original agency item #
- When DNA analysis is reported for samples retained by another discipline in the laboratory, or comparisons are made to profiles reported by another laboratory, the report should reference the previously issued report
 - i.e. "Reference **biological screening** report dated **Month Day, Year** by **Analyst.**"
 - include the issuing laboratory, if other than the AK SCDL.
- Results of presumptive/confirmatory tests, if not previously reported in a biological screening report (in body of report)
 - DNA analysts should report the results of microscopic examinations of swabs when only the smears (submitted in the kit) were examined and reported by a biological screening analyst
- Results, conclusions and opinions for all tested items, based on the DNA typing results
- Results of any statistical analyses performed
- Explanation of why testing was stopped, if appropriate
- Description of analyses performed, including a list of STR loci amplified (this is contained in the Methods section of the report templates for DNA Major Crimes and DNA Property Crimes)
- Description of statistical analyses performed including the methods and loci used in the calculations (this is contained in the Methods section of the report templates for DNA Major Crimes and DNA Property Crimes)
- The disposition of the evidence (this is contained in the Methods section of the report templates for DNA Major Crimes and DNA Property Crimes)
- Signatures of the reporting analyst and the technical reviewer (the analyst should electronically sign the report by setting the milestone to Draft Complete prior to submitting for technical review; only the analyst is required to scan their barcode and enter a pin when signing the report in LIMS)

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For single source samples	Report
Single Source: exclusion	XXX was excluded as the source of DNA detected in this sample.
Single Source: fail to exclude	XXX cannot be excluded as the source of DNA detected in this sample.
Same profile (for 2+ items)	The genetic profiles obtained from these samples were the same.
Single source statistic	<p>Single Source formula</p> <p>The estimated frequency (13 of 13 core loci) of the genetic profile from the above sample(s) is approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Alaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).</p>

For single source and/or mixtures	Report
Unknown Male DNA	A genetic profile from an unknown male individual was obtained from this sample.
Female present	The genetic profile obtained from this sample was consistent with being from an unknown female individual.
Male DNA present	DNA from (a OR at least one) male individual was observed in this sample.
No DNA inconsistent	No DNA inconsistent with XXX was detected in this sample.
DNA consistent	DNA consistent with XXX was detected in this sample
When cannot exclude but don't have all 13 core loci	DNA consistent with XXX was detected at XX of 13 core loci in this sample. Therefore, XXX cannot be excluded as a source of DNA detected in this sample. (change 13 if not all loci yielded data above reporting threshold)

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For mixtures	Report
More than 1 individual	DNA from more than one individual was observed in this sample.
To indicate # of individuals	DNA from at least XX individuals was observed in this sample.
Mixture: exclusion	XXX was excluded as a source of DNA detected in this sample.
Mixture: fail to exclude	XXX cannot be excluded as a source of DNA detected in this sample.
Foreign DNA present	DNA inconsistent with XXX was also detected in this sample.
Complex Mixture	Due to the complexity of the genetic profile obtained from this sample, no meaningful comparisons can be made to known reference samples.
Mixture statistic	Alleles below the reporting threshold may be present at the following loci: XXX. Therefore, population statistics are not reported for these loci.
Mixture statistic	<p>Mixture formula</p> <p>The estimated probability (13 of 13 core loci) of an individual from each of the following population groups contributing to the DNA detected in the above sample(s) is as follows: approximately 1 in XXX quadrillion (Caucasian population), 1 in XXX quadrillion (African-American population), 1 in XXX quadrillion (Athabaskan population), 1 in XXX quadrillion (Inupiat population) and 1 in XXX quadrillion (Yupik population).</p>

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Paternity	Report
Cannot exclude	XXX cannot be excluded as the biological father/mother of XXX. For additional information please contact the DNA Technical Manager Abirami Chidambaram at (907)-269-5621
Excluded	XXX was excluded as the biological father/mother of XXX.
Not Suitable for comparison	Report
Not suitable	The data obtained from this sample was not suitable for comparison (due to XXXXXX).
No profile (No reproducible alleles above 100RFU in at least four core STR loci)	No genetic profile was obtained from this sample.
Non-reproducible	The majority of data in this sample was not reproducible. Therefore, this sample was not suitable for comparison.
No conclusions	Report
Inconclusive	No conclusions are reported as to whether XXX contributed DNA to this sample.
Insufficient	No (other) conclusions are reported for this sample due to insufficient DNA.

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Section 8 Statistical Analysis of STR Data

Statistical analyses will be performed when the genetic typing results fail to exclude an individual(s) as a source of the DNA in a questioned sample and the result is probative. A statistical analysis may not be necessary when an individual is identified as a possible source of DNA in a sample that is intimate to that individual or the result is not germane to the case (i.e. DNA consistent with consent partner detected).

To interpret the significance of a DNA "match" between samples, it is necessary to know the population distribution of alleles at the loci in question. This requires a database containing the frequency of the alleles (observed $\#/2N$) at each of the loci in question. If an allele has been observed five times or less, a value of $5/2N$ is used, where N is equal to the number of individuals tested for the database. The allele frequencies are used to calculate the genotype frequency for each locus.

The allele frequencies for the Athabaskan, Yupik, and Inupiat Alaskan Native populations were calculated using data generated by the Alaska Scientific Crime Detection Laboratory from 101 Athabaskan, 100 Yupik, and 109 Inupiat blood/saliva samples (B. Budowle et al., Population studies on three Native Alaska population groups using STR loci, *Forensic Science International* 129 (2002) 51-57).

The allele frequencies for the Caucasian and African-American populations are from the U.S. Department of Justice, FBI Popstats database. These allele frequencies can be found at the end of this section. The Alaska Scientific Crime Detection Laboratory routinely reports the frequency/probability for all five populations in the DNA Laboratory Report.

Statistical analyses are calculated with the U.S. Department of Justice, FBI Popstats program, using either the Random Match Probability Formula for single source samples or the Mixture Formula for mixed samples. The resultant values may be truncated for reporting, but should never be rounded up (i.e. 34,675,000 may be reported as 34 million or 34.6 million, but not 34.7 million).

8.1 Random Match Probability Formula

To calculate the frequency of a locus genotype, the following formulae are used:

Heterozygotes	$2pq$
Homozygotes	$p^2 + p(1-p)\theta$

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The p and q represent the frequencies of two different alleles. θ (theta) is an empirical measure of population subdivision/substructure or “relatedness”.

For the Caucasian and African-American populations, $\theta=0.01$

For the Alaskan Native populations, $\theta=0.03$

The multi-locus genotype frequency is estimated by multiplying together the genotype frequencies from the different loci. The expected frequency of a DNA profile in a population is the inverse of the multiple locus genotype frequency.

8.2 Combined Probability of Inclusion (CPI; Mixture Formula)

To calculate the probability of a random individual in the population being a contributor to a mixture for a locus, the following formula is used:

$$(p_1 + p_2 + \dots + p_n)^2 = P_{\text{Locus}}$$

The p_1 , p_2 , and p_n are the frequencies of occurrence of the alleles at the locus.

The combined mixture profile probability is calculated by taking the product of the individual locus probabilities. To estimate the number of individuals in a population that could have contributed to the mixture, the inverse of the combined mixture profile frequency is calculated.

8.3 STR Loci and Allele Frequencies used in POPSTATS calculations

D3S1358

Allele	African-American (N=210)	Caucasian (N=203)	Athabaskan (N=101)	Inupiat (N=109)	Yupik (N=100)
<12	0.0119	0.0123	0.0248	0.0229	0.0250
12	0.0119	0.0123	0.0248	0.0229	0.0250
13	0.0119	0.0123	0.0248	0.0229	0.0250
14	0.1214	0.1404	0.1337	0.0275	0.0250
15	0.2905	0.2463	0.4109	0.4541	0.3550
15.2	0.0119	0.0123	0.0248	0.0229	0.0250
16	0.3071	0.2315	0.2574	0.4404	0.5250
17	0.2000	0.2118	0.1436	0.0551	0.0900
18	0.0548	0.1626	0.0545	0.0229	0.0250
19	0.0119	0.0123	0.0248	0.0229	0.0250
>19	0.0119	0.0123	0.0248	0.0229	0.0250

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<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<11	0.0139	0.0128	0.0248	0.0229	0.0250
11	0.0139	0.0128	0.0248	0.0229	0.0250
12	0.0139	0.0128	0.0248	0.0229	0.0250
13	0.0139	0.0128	0.0248	0.0229	0.0250
14	0.0667	0.1020	0.1733	0.1743	0.0800
15	0.2361	0.1122	0.0297	0.1055	0.1300
16	0.2694	0.2015	0.3960	0.1881	0.3100
17	0.1833	0.2628	0.2327	0.2936	0.1350
18	0.1361	0.2219	0.1139	0.1833	0.1200
19	0.0722	0.0842	0.0545	0.0367	0.1250
20	0.0278	0.0128	0.0248	0.0229	0.0250
21	0.0139	0.0128	0.0248	0.0229	0.0250
>21	0.0139	0.0128	0.0248	0.0229	0.0250

D8S1179

<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<8	0.0139	0.0128	0.0248	0.0229	0.0250
8	0.0139	0.0179	0.0248	0.0229	0.0250
9	0.0139	0.0128	0.0248	0.0229	0.0250
10	0.0250	0.1020	0.0297	0.0642	0.0250
11	0.0361	0.0587	0.0248	0.0229	0.0250
12	0.1033	0.1454	0.2079	0.1881	0.0950
13	0.1222	0.3393	0.4555	0.3578	0.4500
14	0.1833	0.2015	0.1980	0.2477	0.3350
15	0.2139	0.1097	0.0941	0.1239	0.0950
16	0.0444	0.0128	0.0248	0.0229	0.0250
17	0.0139	0.0128	0.0248	0.0229	0.0250
>17	0.0139	0.0128	0.0248	0.0229	0.0250

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D18S51

<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<11	0.0139	0.0128	0.0248	0.0229	0.0250
11	0.0139	0.0128	0.0248	0.0229	0.0250
12	0.0583	0.1276	0.1535	0.0688	0.0250
13	0.0556	0.1225	0.2970	0.1514	0.1850
13.2	0.0139	0.0128	0.0248	0.0229	0.0250
14	0.0639	0.1735	0.1287	0.1193	0.1350
14.2	0.0139	0.0128	0.0248	0.0229	0.0250
15	0.1667	0.1276	0.0941	0.3028	0.3000
15.2	0.0139	0.0128	0.0248	0.0229	0.0250
16	0.1889	0.1071	0.1584	0.0367	0.0400
17	0.1639	0.1556	0.0693	0.2477	0.2250
18	0.1306	0.0918	0.0545	0.0275	0.0300
19	0.0778	0.0357	0.0297	0.0459	0.0250
20	0.0556	0.0255	0.0248	0.0229	0.0250
21	0.0139	0.0128	0.0248	0.0229	0.0250
21.2	0.0139	0.0128	0.0248	0.0229	0.0250
22	0.0139	0.0128	0.0248	0.0229	0.0250
>22	0.0139	0.0128	0.0248	0.0229	0.0250

D13S317

<i>Allele</i>	<i>African-American (N=179)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<8	0.0140	0.0128	0.0248	0.0229	0.0250
8	0.0363	0.0995	0.0693	0.1055	0.1100
9	0.0279	0.0765	0.1881	0.1330	0.1050
10	0.0503	0.0510	0.2277	0.2661	0.4250
11	0.2874	0.3189	0.2772	0.3853	0.2700
12	0.4832	0.3087	0.1881	0.0826	0.0900
13	0.1257	0.1097	0.0396	0.0229	0.0250
14	0.0363	0.0357	0.0248	0.0229	0.0250
15	0.0140	0.0128	0.0248	0.0229	0.0250
>15	0.0140	0.0128	0.0248	0.0229	0.0250

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FGA

<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<18	0.0139	0.0128	0.0248	0.0229	0.0250
18	0.0139	0.0306	0.0248	0.0229	0.0250
18.2	0.0139	0.0128	0.0248	0.0229	0.0250
19	0.0528	0.0561	0.0248	0.1009	0.2000
19.2	0.0139	0.0128	0.0248	0.0229	0.0250
20	0.0722	0.1454	0.0693	0.0459	0.0350
20.2	0.0139	0.0128	0.0248	0.0229	0.0250
21	0.1250	0.1735	0.1634	0.0505	0.0250
21.2	0.0139	0.0128	0.0248	0.0229	0.0250
22	0.2250	0.1888	0.0941	0.1651	0.2150
22.2	0.0139	0.0128	0.0248	0.0229	0.0250
22.3	0.0139	0.0128	0.0248	0.0229	0.0250
23	0.1250	0.1582	0.1287	0.1147	0.1050
23.2	0.0139	0.0128	0.0248	0.0229	0.0250
24	0.1861	0.1378	0.2178	0.2248	0.1200
24.2	0.0139	0.0128	0.0248	0.0229	0.0250
24.3	0.0139	0.0128	0.0248	0.0229	0.0250
25	0.1000	0.0689	0.1881	0.1514	0.2100
26	0.0361	0.0179	0.1089	0.1101	0.0700
26.2	0.0139	0.0128	0.0248	0.0229	0.0250
27	0.0222	0.0128	0.0248	0.0275	0.0250
28	0.0167	0.0128	0.0248	0.0229	0.0250
29	0.0139	0.0128	0.0248	0.0229	0.0250
30	0.0139	0.0128	0.0248	0.0229	0.0250
>30	0.0139	0.0128	0.0248	0.0229	0.0250

TH01

<i>Allele</i>	<i>African-American (N=210)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<5	0.0119	0.0123	0.0248	0.0229	0.0250
5	0.0119	0.0123	0.0248	0.0229	0.0250
6	0.1095	0.2266	0.1535	0.0229	0.0850
7	0.4405	0.1724	0.6089	0.8762	0.7700
8	0.1857	0.1256	0.0594	0.0229	0.0550
8.3	0.0119	0.0123	0.0248	0.0229	0.0250
9	0.1452	0.1650	0.0644	0.0275	0.0250
9.3	0.1048	0.3054	0.1139	0.0596	0.0700
10	0.0143	0.0123	0.0248	0.0229	0.0250
>10	0.0119	0.0123	0.0248	0.0229	0.0250

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D21S11

<i>Allele</i>	<i>African-American (N=179)</i>	<i>Caucasian (N=196)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<24.2	0.0140	0.0128	0.0248	0.0229	0.0250
24.2	0.0140	0.0128	0.0248	0.0229	0.0250
24.3	0.0140	0.0128	0.0248	0.0229	0.0250
26	0.0140	0.0128	0.0248	0.0229	0.0250
27	0.0615	0.0459	0.0248	0.0229	0.0250
28	0.2151	0.1658	0.0297	0.0229	0.0250
29	0.1899	0.1811	0.1683	0.2982	0.3150
29.2	0.0140	0.0128	0.0248	0.0229	0.0250
30	0.1788	0.2321	0.4257	0.1978	0.2000
30.2	0.0140	0.0383	0.0248	0.0229	0.0250
30.3	0.0140	0.0128	0.0248	0.0229	0.0250
31	0.0922	0.0714	0.0545	0.1055	0.0650
31.2	0.0754	0.0995	0.1238	0.1881	0.2000
32	0.0140	0.0153	0.0248	0.0229	0.0250
32.1	0.0140	0.0128	0.0248	0.0229	0.0250
32.2	0.0698	0.1122	0.1238	0.1284	0.1100
33	0.0140	0.0128	0.0248	0.0229	0.0250
33.2	0.0335	0.0306	0.0594	0.0413	0.0750
34	0.0140	0.0128	0.0248	0.0229	0.0250
34.2	0.0140	0.0128	0.0248	0.0229	0.0250
35	0.0279	0.0128	0.0248	0.0229	0.0250
35.2	0.0140	0.0128	0.0248	0.0229	0.0250
36	0.0140	0.0128	0.0248	0.0229	0.0250
>36	0.0140	0.0128	0.0248	0.0229	0.0250

D5S818

<i>Allele</i>	<i>African-American (N=180)</i>	<i>Caucasian (N=195)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<7	0.0139	0.0128	0.0248	0.0229	0.0250
7	0.0139	0.0128	0.1832	0.0826	0.0650
8	0.0500	0.0128	0.0248	0.0229	0.0250
9	0.0139	0.0308	0.0248	0.0229	0.0250
10	0.0639	0.0487	0.0743	0.0688	0.1150
11	0.2611	0.4103	0.5050	0.3578	0.4800
12	0.3556	0.3539	0.1436	0.2936	0.2350
13	0.2444	0.1462	0.0743	0.1514	0.0850
14	0.0139	0.0128	0.0248	0.0229	0.0250
15	0.0139	0.0128	0.0248	0.0229	0.0250
>15	0.0139	0.0128	0.0248	0.0229	0.0250

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D7S820

<i>Allele</i>	<i>African-American (N=210)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<6	0.0119	0.0123	0.0248	0.0229	0.0250
6	0.0119	0.0123	0.0248	0.0229	0.0250
7	0.0119	0.0172	0.0248	0.0229	0.0250
8	0.1738	0.1626	0.1485	0.2615	0.3650
9	0.1571	0.1478	0.1089	0.1239	0.0650
10	0.3238	0.2906	0.2673	0.0780	0.1200
10.1	0.0119	0.0123	0.0248	0.0229	0.0250
11	0.2238	0.2020	0.2426	0.3395	0.2750
11.3	0.0119	0.0123	0.0248	0.0229	0.0250
12	0.0905	0.1404	0.2178	0.1743	0.1400
13	0.0191	0.0296	0.0248	0.0229	0.0350
14	0.0119	0.0123	0.0248	0.0229	0.0250
>14	0.0119	0.0123	0.0248	0.0229	0.0250

D16S539

<i>Allele</i>	<i>African-American (N=209)</i>	<i>Caucasian (N=202)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<8	0.0120	0.0124	0.0248	0.0229	0.0250
8	0.0359	0.0198	0.0248	0.0229	0.0250
9	0.1986	0.1640	0.1337	0.0688	0.1800
10	0.1101	0.0668	0.2921	0.0826	0.0800
11	0.2943	0.2723	0.2772	0.6147	0.3550
12	0.1866	0.3391	0.2426	0.2202	0.3400
13	0.1651	0.1634	0.0446	0.0229	0.0400
14	0.0120	0.0322	0.0248	0.0229	0.0250
15	0.0120	0.0124	0.0248	0.0229	0.0250
>15	0.0120	0.0124	0.0248	0.0229	0.0250

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TPOX

<i>Allele</i>	<i>African-American (N=209)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<6	0.0120	0.0123	0.0248	0.0229	0.0250
6	0.0861	0.0123	0.0248	0.0229	0.0250
7	0.0215	0.0123	0.0248	0.0229	0.0250
8	0.3684	0.5443	0.2178	0.2477	0.2100
9	0.1818	0.1232	0.0248	0.0413	0.1200
10	0.0933	0.0370	0.0248	0.0229	0.0250
11	0.2249	0.2537	0.4010	0.5184	0.3250
12	0.0239	0.0394	0.3515	0.1789	0.1100
13	0.0120	0.0123	0.0248	0.0229	0.0250
>13	0.0120	0.0123	0.0248	0.0229	0.0250

CSF1PO

<i>Allele</i>	<i>African-American (N=210)</i>	<i>Caucasian (N=203)</i>	<i>Athabaskan (N=101)</i>	<i>Inupiat (N=109)</i>	<i>Yupik (N=100)</i>
<6	0.0119	0.0123	0.0248	0.0229	0.0250
6	0.0119	0.0123	0.0248	0.0229	0.0250
7	0.0429	0.0123	0.0248	0.0229	0.0250
8	0.0857	0.0123	0.0248	0.0229	0.0250
9	0.0333	0.0197	0.1040	0.0642	0.0350
10	0.2714	0.2537	0.1683	0.2936	0.3550
10.3	0.0119	0.0123	0.0248	0.0229	0.0250
11	0.2048	0.3005	0.2129	0.1973	0.1400
12	0.3000	0.3251	0.4257	0.4312	0.3600
12.1	0.0119	0.0123	0.0248	0.0229	0.0250
13	0.0548	0.0714	0.0792	0.0229	0.0300
14	0.0119	0.0148	0.0248	0.0229	0.0300
15	0.0119	0.0123	0.0248	0.0229	0.0450
>15	0.0119	0.0123	0.0248	0.0229	0.0250

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Section 9 Review of Data

This section supplements information contained in the Laboratory Quality Assurance Manual and the DNA Quality Assurance Manual.

9.1 Review of Casework Analyzed In-House

All case reports issued by the Forensic Biology discipline, and all supporting documentation that is part of the case record or the central log, will be subjected to a technical review and an administrative review. When a case has been through technical and administrative review, the report will be issued in accordance with laboratory policies and procedures. Genetic profiles suitable for CODIS will be uploaded in accordance with CODIS policies and procedures.

9.1.1 Technical and Administrative Review

Technical review of casework will be conducted by a second qualified analyst. The analyst and the reviewer may consult a third qualified analyst, if necessary, when there is a disagreement on how to report a result. If the analysts are unable to come to an agreement, the DNA Technical Manager will be consulted to make the final decision.

Discipline checklists are used to document completion of the individual components of the technical and administrative reviews. The completion of the final laboratory report, technical review and administrative review are tracked electronically in LIMS.

9.2 Review of Offender Database Samples Analyzed In-House

Genetic profiles and all supporting documentation generated in the course of analysis (for entry into CODIS or hit confirmation) will be subjected to a technical review. Technical review of offender samples will be conducted by a second qualified DNA analyst, in accordance with the FBI QAS Guidelines and the laboratory and DNA Quality Assurance Manual. The analyst and the reviewer may consult a third qualified DNA analyst, if necessary, when there is a disagreement on how to report a result. If the analysts are unable to come to an agreement, the DNA Technical Manager will be consulted to make the final decision.

A review checklist is used to document completion of the individual components of the technical and administrative review. When the technical review is complete, offender samples may be uploaded to CODIS. Detailed procedures for upload and resolution of any resulting database matches are contained in the laboratory's CODIS manual.

9.3 Review of Outsourced Offender Database Samples

The laboratory does not currently use a vendor laboratory for DNA analysis. The laboratory may perform a technical review of data generated by a vendor laboratory under contract with an Alaska law enforcement agency.

Please refer to the DNA Quality Assurance Manual for additional information.

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Section 10 Laboratory Maintenance

The Forensic Biology Staff are responsible for the housekeeping in the laboratory and for the routine maintenance of equipment and instruments (as described in the Forensic Biology Work Instructions). These tasks are designated to the unit technician, or another unit member when the technician is unavailable. Log sheets for maintenance and housekeeping are completed as appropriate.

- Receipt of packages and logging of chemicals/reagents.
 - Indicate date received on packing slip, initial and provide to the unit supervisor.
 - Unpack contents, label with date received and initials, store them in the proper location, record in logbook
 - If there are multiples of the same lot number (i.e. kits) then label the boxes or reagents with sequential numbers
 - Label with "needs verification" stickers and note on board that verification is required (if appropriate)
- Clean laboratories weekly, wiping down counters, computers, centrifuges, phones, door handles, etc. with 10% bleach. Each analyst is responsible for bleaching his/her own personal computer and workspace.
- UV PCR set-up hoods weekly (for at least four hours)
- Wipe down equipment/instruments weekly.
- Reboot instrument computers weekly.
- Sweep and mop floors monthly. Log cleaning in on sheets provided in each lab.
- Perform weekly, monthly, and semi-annual maintenance on instruments. These tasks are detailed in the Forensic Biology Work Instructions document.
- Defragment instrument computer hard drives monthly. Each analyst is responsible for his/her personal computer(s).
- Put away clean laboratory dishes as needed.
- Keep both labs well stocked and note (write on board) reagents and supplies that need to be ordered.
- Replenish reagents on genetic analyzers, as needed.
- Autoclave water as needed. Make sure that new kits/reagents are verified in a timely fashion.

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DNA STR Results Table

Laboratory Case #
 Batch:

Analyst:

Item #					
Description/ Locus					
D3S1358					
TH01					
D21S11					
D18S51					
Penta E ^{NS}					
D5S818					
D13S317					
D7S820					
D16S539					
CSF1PO					
Penta D ^{NS}					
Amelogenin					
vWA					
D8S1179					
TPOX					
FGA					

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Notes:

1. The Amelogenin locus indicates gender: X = Female; XY = Male
2. "NS" indicates data not used in statistical analysis
3. "NR" indicates data not reported
4. "*" indicates data below reporting threshold (100 RFU)
5. () indicates minor component alleles
6. [] indicates Major/Minor components not separated
7. "A/TA" indicates artifact (e.g. stutter) or true allele
8. Genetic data unsuitable for comparison may not be included in the table

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Biological Screening Review Checklist

		Technical Review	Administrative Review
LAB NUMBER:			
ANALYST:			
Date Submitted for Review:			
Technical Review Started:	Reviewer:		
Administrative Review Started:	Reviewer:		
Pages are numbered correctly, lab case #, item # and analyst initials are on each page			
Requesting agency, agency case #, lab case #, and officer's name are correct			
Item numbers / packaging / descriptions on report / notes are consistent with RLS/LIMS			
The type of examination (visual, stereoscopic, ALS) and testing performed is documented in notes			
Item descriptions are consistent with clothing/evidence image present (if applicable)			
The location of all chemical testing performed is documented in the notes (if applicable)			
All isolated stains/samples are documented and numbered correctly (if applicable)			
Verification reviews conducted are documented in the notes (if applicable)			
The location and disposition of all trace evidence is documented			
Worksheets contain all lot #s and expiration dates for all reagents used			
The date evidence was opened and/or sealed is documented			
Retained items created in LIMS; all retained and unexamined items HELD in LIMS			
The "FUTURE TECH" flag has been tripped for the case, if applicable (samples suitable for Y-STR)			
Check grammar/spelling/punctuation in report			
Report signed in LIMS			
Results/conclusions/opinions are given for each item tested			
Conclusions/opinions drawn from results comply with laboratory guidelines			
Conclusions/opinions drawn from results are supported by documentation in the notes			
All case related notes and attached/scanned documents are present			
Technical reviewer is in review history for each page of the bench notes			
SOPs are linked to request in LIMS			
Chain of Custody for all tested items can be tracked through RLS and LIMS			
Assign DNA holding to DNA Supervisor			
VERIFICATION REVIEW	Analyst:	Date:	
Semen/ABAcad® p30 Test			
Species/ABAcad® HemaTrace®			
Hairs - Stereoscopic			

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STR Casework Review Checklist

Date: _____
Technical Review Started: _____
Administrative Review Started: _____
Lab Number: _____

Analyst:	Tech. Review:	Admin. Review:

DNA CASEWORK REPORT AND BENCH NOTES

Pages are numbered correctly, case # and analyst initials are on each page, report dated			
Requesting agency, agency case #, crime lab case #, and officer's name are correct			
Chain of Custody for all tested items can be tracked through RLS and LIMS			
Item numbers and descriptions on report / DNA worksheet are consistent with RLS/LIMS			
Packaging / extraction procedure used for all items is documented on worksheets			
Q-PCR results documented on DNA worksheet			
Screening results on DNA worksheets are accounted for in the report, if appropriate			
Disposition of sample extracts/slides documented; item created in LIMS (if appropriate)			
Technical Reviewer checked DNA worksheet: initialed and dated			
Technical review of all electropherograms: initialed and dated			
PP16 printouts for all questioned and reference samples are present in bench notes			
Peak patterns on the electropherograms are consistent with the STR results table (if applicable)			
The correct DNA profile and descriptive information is entered into Popstats			
Popstats printouts are included in the case file			
Popstats DNA profile frequencies are consistent with those shown on the report			

Check Grammar/Spelling in DNA report			
Results/conclusions/opinions are given for each tested item			
Conclusions/opinions drawn from results comply with laboratory guidelines			
Initials and date of technical review(er) are on each page of the bench notes			
Verify content of Methods and Disposition sections of report			
Stats are recorded in LIMS			
SOPs are linked to request in LIMS			
Disposition of evidence in LIMS is appropriate (HOLD or not)			

CENTRAL LOG FOLDER LOCATION

Central Log Folder location documented in LIMS			
Q-PCR plate set-up is documented			
Q-PCR standard curve (Mx3000P) printout: Results are acceptable.			
Q-PCR Initial Temp and Quantity is documented by Mx3000P printout.			
Amplification volumes are documented.			
ICS checked by Technical Reviewer			
Technical Reviewer checked Controls and Standards sheet: initialed and dated.			
Disposition of blank extracts documented; item created in LIMS (if appropriate)			
PP16 printouts for negative control(s): Results are acceptable.			
PP16 printouts for reagent blank(s): Results are acceptable.			
PP16 printouts for positive control(s): correct DNA profile obtained.			
PP16 printouts for all appropriate Allelic Ladders.			
Worksheets contain all lot #s and expiration dates for all reagents used.			

List of eligible profiles to be entered into SDIS/NDIS (include specimen category)			
Correct profile(s) and agency information entered in CODIS			
Appropriate specimen category selected			
Source ID updated if applicable			
Offender/arrestee laboratory comments updated in LIMS, if applicable			

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STR Single Offender Review Checklist

Date: _____
Technical Review Started: _____
Administrative Review Started: _____
Lab Number: _____

Analyst:	Tech. Review:
----------	---------------

OFFENDER FOR DNA DATABASE OR CODIS CONFIRMATION

Offender number(s) and analyst initials are on each page		
Offender number(s) and name(s) on DNA worksheet are consistent with LIMS		
Packaging / extraction procedure is documented on DNA worksheet		
Q-PCR results documented on DNA worksheet		
Disposition of offender extract(s) documented		
Technical Reviewer checked DNA worksheet: initialed and dated		
Technical review of all electropherograms: initialed and dated		
Results (i.e. profile verified) given for each tested item		
Initials and date of technical review(er) are on each page of the bench notes		
SOPs are linked to request in LIMS		

CENTRAL LOG FOLDER LOCATION:

Central Log Folder location documented in LIMS		
Q-PCR plate set-up is documented		
Q-PCR standard curve Mx3000P printout: Results are acceptable.		
Q-PCR Initial Template Quantity is documented by Mx3000P printout.		
Amplification volumes are documented.		
ICS checked by Technical Reviewer		
Technical Reviewer checked Controls and Standards sheet: initialed and dated.		
Disposition of blank extracts documented; items created in LIMS (if appropriate)		
PP16 printouts for negative control(s): Results are acceptable.		
PP16 printouts for reagent blank(s): Results are acceptable.		
PP16 printouts for positive control(s): correct DNA profile obtained.		
PP16 printouts for all appropriate allelic ladders.		
Worksheets contain all lot #s and expiration dates for all reagents used.		
List of eligible profiles to be entered into SDIS/NDIS (include specimen category)		
Correct profile(s) and agency information entered in CODIS		
Appropriate specimen category selected		
Source ID updated if applicable		
Offender/arrestee laboratory comments updated in LIMS, if applicable		

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STR Offender Batch Review Checklist

Date: _____
 Technical Review Started: _____
 Administrative Review Started: _____
 Database Batch Number: _____

Analyst:	Tech. Review:	Admin. Review:

Database batch # and analyst's initials on each page			
Reagent worksheet complete: analysis dates, instruments, # samples, reagent lot # and expiration dates, raw data folder(s) and project name(s)			
Batch worksheet complete: well #s, sample IDs, upload eligibility, specimen category and analysis range.			
Technical Reviewer checked batch worksheet (inc. reagents): initialed and dated each page			
ILS labeled correctly (80-550) for all passing samples			
Allelic Ladder(s): correct allele calls obtained ¹			
Positive control(s): correct DNA profile obtained ¹			
Negative control(s): results are acceptable ¹			
Reagent blank(s): results are acceptable ¹			
Check for consistency between NO EXPORT samples on paperwork and in project			
Confirm appropriateness of NO EXPORT status			
Technical review of passing samples: ≤3 problem control loci, RFU 75-250, appropriate allele edits, OL allele documentation ¹			
Correct specimen categories are assigned			
Confirm specimen category is either convicted offender or arrestee for passing samples (check for agreement between paperwork and project)			
Samples for SDIS only are flagged on paperwork			

¹ includes verification of ILS and controls for reworked samples

Eligible profiles uploaded into SDIS (in the Offender Index)			
Reconciliation report: #samples uploaded consistent with batch worksheet			
Problem samples corrected and documentation attached			
Duplicate offender search performed and all high stringency matches reconciled			
AK new moderate stringency search performed (only after previous step completed)			
AK new moderate stringency search may be completed by analyst or administrative reviewer			

Performed by CODIS Administrator

Upload to NDIS performed			
Reconciliation report received; consistent with # samples uploaded			

NOTES:

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CODIS Match/Hit Review Checklist

Date Submitted for Review:: _____	Analyst:	Reviewer:
Administrative Review Started:: _____		
Lab Number:: _____		

Match/Hit Letter Checklist (all communications to AK law enforcement)

Cases related in LIMS		
Match disposition updated in CODIS, source ID updated		
Match confirmed: including profile verification if necessary		
Case Activities: correct hit disposition, investigation aided indicated (if appropriate), match date as start date		
Agency contact requested regarding status of case and probative nature of match		
Identity of offender attempted by latent prints; indicated in letter		
Qualifying offense of offender verified if AK offender; comment added to case synopsis		
Match level indicated in body of letter (SDIS or NDIS)		
Correct agency/lab, agency/lab rep., agency/lab case#, item#, item description, and contact information in letter		
Correct name and DOB of offender (and state, if applicable)		
New sample requested, if applicable		
Confirm content of last known whereabouts, if applicable.		

Interstate CODIS Confirmation Checklist (all communications to out-of-state laboratory)

Match disposition updated in CODIS, source ID updated		
Match confirmed: including profile verification if necessary		
Case Activities: correct hit disposition, investigation aided indicated (if appropriate), match date as start date		
Agency contact requested regarding status of case and probative nature of match		
Identity of offender attempted by latent prints (if AK offender); indicated in letter		
Qualifying offense of offender verified if AK offender; comment added to case synopsis		
Match level indicated in body of letter (SDIS or NDIS)		
Correct agency/lab, agency/lab rep., agency/lab case#, match ID, item#, item description, and contact information in letter		
Correct name and DOB of offender (and other pertinent identifying information, if provided)		
New sample requested, if applicable		
Confirm content of last known whereabouts, if applicable.		

NOTES:

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STR Review Checklist for Vendor Lab

Date: _____
SCDL Lab Number: _____
Vendor Laboratory: _____
Vendor Lab Number: _____
Agency: _____
Agency #: _____
Technology: _____
Platform: _____
Amplification kit: _____

Tech Review:	CODIS Review:
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PP16 printouts for all appropriate Allelic Ladders: obtained expected ILS and allelic peaks		
PP16 printouts for positive control: obtained expected ILS peaks and DNA profile		
PP16 printouts for negative control: results are acceptable (including ILS)		
PP16 printouts for reagent blanks: results are acceptable (including ILS)		

Screening results on DNA worksheets are accounted for in the report, if appropriate		
Raw or analyzed data provided for each tested item		
Technical review of all electropherograms (including ILS); initialed and dated by SCDL		
Peak patterns on the electropherograms are consistent with the table (if applicable)		

Results/conclusions/opinions are given for each tested item		
Conclusions/opinions drawn from results are supported by the genetic data		
Initials and date of technical review (if any) are on each page		

List of eligible profiles to be entered into CODIS/NDIS (include specimen category)		
Correct profile(s) and agency information entered in CODIS		
Appropriate specimen category selected		
Source ID updated if applicable		

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Casework Extraction Lab Cleaning Log

Date /Initial	Task Completed	Comments
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
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	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	

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PCR Prep Lab Cleaning Log

Date /Initial	Task Completed	Comments
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
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	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization	

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PCR Lab Cleaning Log

Date /Initial	Task Completed	Comments
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> 9.3,10 resolution, water wash, flush trap <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> ABI 3130xl(s) wiped down <input type="checkbox"/> 9.3,10 resolution, water wash, flush trap <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> ABI 3130xl(s) wiped down <input type="checkbox"/> 9.3,10 resolution, water wash, flush trap <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> ABI 3130xl(s) wiped down <input type="checkbox"/> 9.3,10 resolution, water wash, flush trap <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	
	<input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> ABI 3130xl(s) wiped down <input type="checkbox"/> 9.3,10 resolution, water wash, flush trap <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual	

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Appendix A Revision History

FBP2012 R0 Page	FBP2011 R0 page	Location	Revision made
		Entire document	Changed "Issued" to "Approved" in footer
	1	Document Structure	Added note to indicate sections of manual approved by Biological Screening Technical Lead.
2	2	Section 1.1	INSERTED "Critical reagents, as specified by the FBI DNA QA Standard 9, require testing on established samples before use on evidentiary or casework samples. Using a positive control() i.e. an established sample(s) to assess the performance of a critical reagent provides a basis for the minimum acceptable performance that can be reasonably expected from that critical reagent based on results of previous testing on the established sample(s). A critical reagent that performs below the minimum acceptable level will be rejected and not used on evidentiary or casework samples. Critical reagents will also be tested using a negative control sample(s) or reagent blank(s) which determine if there is any contamination in the reagent being tested. If the negative control fails – i.e. contamination is detected in the test results, the reagent will be rejected and not used on evidentiary or casework samples."
3	3	Section 1.2	UPDATED "For chemicals/reagents received without a manufacturer's expiration date : Please refer to the DNA QA manual".
4	4	Section 1.2	DELETED " Reagents and chemicals may be re-verified and labeled with the re-verification date to indicate that the item is suitable for use through the new expiration date. Re-verification must be approved in advance by the DNA Technical Manager. This approval will be

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			documented in the form of an email or memorandum maintained with the other verification paperwork.
5	-	Section 1.4	<p>Updated:</p> <p>NOTE: Vendor supplied standard samples / positive control samples such as 9547A that are sent with PCR amplification kits may be discontinued or substituted at vendors' discretion. Analyst bench notes and batch central log information will indicate the identity of positive control samples used in analysis. Kit component information in the chemicals and reagents section(s) of manuals will be updated as required when manuals are revised.</p>
9	8	Section 2.2	<p>Revised procedure to reflect that smear slides received in kits will not be stained and examined. Swabs will always be extracted and examined for the presence of spermatozoa.</p>
9	8	Section 2.3	<p>Removed "Swabs collected from the external genital area may be retained for saliva/foreign DNA in cases of alleged or possible oral assault."</p>
9	9	Section 2.3	<p><u>“Samples present in the Miscellaneous Swabs Saliva envelope or any other envelope labeled as a possible saliva, breast swab, bite mark, or licked/sucked area will be retained as a separate item of evidence for further analysis.</u></p> <p><u>Samples present in the Miscellaneous Swabs DNA envelope or any other envelope labeled as a possible contact/touch or skin cell sample may be retained as a separate item of evidence for further analysis.”</u></p> <p>Changed to</p>

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			<p><u>“Samples present in the Miscellaneous Swabs Saliva envelope, the Miscellaneous Swabs DNA envelope, or any other envelope labeled as containing a sample with possible foreign saliva or skin cells, may be suitable for DNA testing.”</u></p>
10	9	Section 2.4	<p>Removed “Samples from the external genitalia area include the Mons Pubis/Labia Majora, Labia Minora, Introitus, Perineum, Anus, Penis and Scrotum.”</p>
10	9	Section 2.4	<p>“Samples may be retained for saliva in cases of alleged or possible oral assault (as indicated).”</p> <p>Changed to</p> <p>“Occasionally, external genitalia samples may be tested for foreign DNA, even when the presence of blood and/or spermatozoa is not indicated.”</p>
10	9	Section 2.4	<p>Removed “prior to isolation” from “Penile swabs obtained from the victim/suspect within 24 hours of the offense may be examined for the presence of blood, epithelial cells or spermatozoa (useful in cases involving multiple suspects or to help establish recent contact) prior to isolation.”</p>
10	9	Section 2.4	<p>Removed “packaging and/or to the” from “The analyst will add any relevant case information to the packaging and/or to the notes section in JusticeTrax (Section 2.12).”</p>
10	9	Section 2.5	<p>Removed “Due to the limited quantity of material recovered in most cases, the presence of potential biological evidence suitable for DNA analysis will be given priority over other biological and trace evidence examinations. If the case scenario suggests the presence of spermatozoa is likely, the analyst will make a note on the retained items packaging and in the notes section in</p>

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			JusticeTrax (Section 2.12)”
10	9	Section 2.5	Added “The analyst will add any relevant case information to the notes section in JusticeTrax (Section 2.12).”
10	10	Section 2.6	<p>“If spermatozoa and/or nucleated epithelial cells are present, create a new item of evidence that includes the swabs and prepared slides. If spermatozoa and/or nucleated epithelial cells are not present, the swabs and slides/smears may be packaged with the original item.”</p> <p>Changed to</p> <ul style="list-style-type: none"> • “Create a new item of evidence that includes the swabs and prepared slides.”
11	10	Section 2.7	Removed “(including a scale)” from the first bulleted statement
11	10	Section 2.7	<p>“Swab the area(s) of interest. If sampling clothing, swab the entire interior surface of the garment.”</p> <p>Changed to</p> <p>“Swab the area(s) of interest (those likely to have the most contact with bare skin).”</p>
11	10-11	Section 2.8	Removed entire section and re-numbered remaining subsections.
12	12	Section 2.9 (formerly 2.10)	<p>“Suspected blood stains, whether visible to the naked eye or detected with the aid of an alternate light source, can be tested for peroxidase-like activity with Phenolphthalein reagent and hydrogen peroxide.”</p> <p>Changed to</p> <p>“Suspected blood stains can be tested for peroxidase-like activity with Phenolphthalein reagent and hydrogen peroxide.”</p>
13	13	Section 2.9	“Stains testing positive to this presumptive

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		(formerly 2.10)	test for blood may be further characterized as to their origin using Human Hemoglobin analysis by immunoassay to determine species.” Changed to “Stains testing positive to this presumptive test for blood may be further characterized as to their origin (human or higher primate) using Human Hemoglobin analysis by immunoassay.”
14	14	Section 2.10 (formerly 2.11)	Take detailed notes describing each item and its packaging. Include the condition of the item, nature and location of the staining, and any damage observed. When practical, digital images of items (with a scale) should be included in the bench notes. Changed to Take detailed notes describing each item and its packaging. When practical, digital images of items should be included in the bench notes.
14	14	Section 2.11 (formerly 2.12)	Removed “Scotch tape, a MACO® label (3/4" X 1"), or any other suitable label or tape may be used.”
15	15	Section 2.11 (formerly 2.12)	Removed Figure 3 and re-numbered subsequent figures.
15	15	Section 2.11 (formerly 2.12)	“Label each envelope with the lab number, unique identifier (item #), a brief description, your initials, and a biohazard label (Figure 3).” Changed to “Label each envelope with the lab number, unique identifier (item #), a brief description, your initials, a biohazard label (when appropriate), and a LIMS barcode (Figure 3).”
16	16	Section 2.11 (formerly 2.12)	Removed “Place a biohazard label and the LIMS bar-coded label on the envelope

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			(Figure 4).”
17	17	Section 2.11 (formerly 2.12)	Added “Typically, entire assault kits will be retained by the laboratory and itemization of contents in LIMS is not necessary.”
37	37	Section 6.2.1	REPLACED "If no interpretable profile is obtained with any suitable positive amplification control(s), then a positive amplification control and a designated percentage of the samples in the run shall be re-amplified and re-typed. <ul style="list-style-type: none"> • 5% of the samples for a database batch • 10% of the samples for a casework batch" WITH “All casework samples in a batch will be re-analyzed if only one positive control sample was used in that batch and this control fails. The laboratory will routinely include at least 5 positive control samples in every casework batch in order to prevent reanalysis of samples that are limited in DNA content. If at least one of these 5 positive control samples produces acceptable and expected results, the batch will not be reanalyzed. A positive control will be defined as a single source sample whose genetic profile was previously determined and from which a full profile was developed. The positive control may be a previously tested convicted offender sample or a buccal swab freshly obtained from laboratory personnel whose genetic profile(s) was/were previously determined and documented”.
64	63	Section 7.1	“All pages in the case record and central log record contain the case number, analyst’s initials, item # (if appropriate, i.e. digital images) and page number (page X of Y) indicating the total number of pages in the record.” Changed to “All pages in the case record and central

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			log record contain the case number, analyst's initials, item # (if appropriate, i.e. digital images) and page number (page X). The total number of pages is indicated by marking the last page as "last page" or "final page".
90	88	Biological Screening Review Checklist	Retained items created in LIMS with correct descriptions (HOLDS) Changed to Retained items created in LIMS; all retained and unexamined items HELD in LIMS
91	89	STR Casework Review Checklist	Added "Analyst:" "Tech Review:" and "Admin Review:" to top of columns in checklist
97	95	Casework Extraction Lab #1 Cleaning Log	Renamed "Casework Extraction Lab Cleaning Log" and removed check box for PCR hood UV sterilization
98	96	Casework Extraction Lab #2 Cleaning Log	Renamed "PCR Prep Lab Cleaning Log" and removed check boxes for EZ1 maintenance
--	97	CODIS Lab Cleaning Log	Removed from document

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